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Analytical Chemistry Division (839)

Willie E. May, Chief

Division Overview

I. Introduction

The Analytical Chemistry Division is one of five Divisions in the Chemical Science and Technology Laboratory, National Institute of Standards and Technology. The Division has approximately 100 scientists, technicians, and administrative/clerical support staff and an annual budget of about \$15M of which about \$6M supports programs for other Federal and State Government Agencies and/or American industry on a cost reimbursable basis.

The Division serves as the Nation's reference laboratory for chemical measurements and standards to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and provide quality assurance for chemical measurements used for assessing and improving public health, safety, and the environment. The Division maintains world-class metrologically based core competencies in:

- Analytical Mass Spectrometry
- Analytical Separation Science
- Atomic and Molecular Spectroscopy
- Chemical Sensing Technology
- Classical and Electroanalytical Methods
- Gas Metrology
- Nuclear Analytical Methods
- Microanalytical Technologies

These competencies provide the capability to carry out the Division's broad mission and the flexibility to respond to changing and evolving national priorities. These core competencies reside in five Groups: [1] Spectrochemical Methods, [2] Organic Analytical Methods, [3] Gas Metrology and Classical Methods, [4] Molecular Spectrometry and Microfluidic Methods and [5] Nuclear Analytical Methods. The skills and knowledge derived from laboratory-based research concerning the phenomena that underpin the measurement of chemical species in a broad spectrum of matrices are applied to the development and critical evaluation of measurement methods of known accuracy and uncertainty. These internationally-recognized reference methods support the Division's infrastructure for providing traceability of chemical measurements used in programs of National/International importance through:

- Standard Reference Materials
- NIST Traceable Reference Materials (NTRMs)
- Measurement Quality Assurance Programs in critical areas
- Comparisons of NIST chemical measurement capabilities and standards with other National Metrology Institutes.

II. Division Project Areas in Support of Chemical Science and Technology Laboratory Programs

Reference Methods and Standards for Healthcare

The objective of this project is to develop and maintain the measurements and standards infrastructure to facilitate accurate decision-making regarding the diagnosis, treatment, and prevention of diseases. Measurements are responsible for 10% - 15% of the \$1.3 T annual costs of healthcare in the United States. A significant portion (25% - 30%) of health-related measurements are performed for non-diagnostic reasons (re-tests, error prevention and detection). Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in health care costs. Project drivers are therefore, measurement reliability as it impacts healthcare costs and medical decision-making, regulatory requirements, and international trade and competitiveness-related issues.

Project components include [1] Reference Methods for selected health status markers (electrolytes, small organic markers, toxic and/or speciated metals, protein-based markers) [2] Standard Reference Materials (pure primary chemical standards, optical filter standards for instrument calibration/assessment, body fluid-based materials) , [3] interactive measurement quality assessment activities, [4] strategic international comparison exercises

NIST works with other government agencies (e.g., CDC, NCI, NIH), professional organizations (e.g., AACC, CAP, NCCLS), and the private sector (e.g., AdvaMED , Mayo Clinic) to prioritize measurement and standards needs.

Measurement Methods and Standards for Forensic Analyses

Forensic chemical analyses have become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications.

Project activities address measurement method and standards issues related to alcohol and drugs of abuse testing, human identity testing based (DNA and chemical profiles in hair), crime scene investigations (gunshot and explosive residues, teleforensics), chemical and biological weapons detection.

Project priorities are driven by input from the U.S. Departments of Justice and Defense.

Measurement Methods and Standards for Nutrients, Contaminants, and Adulterants in Foods

The integrity of the nation's food supply is important for public health and safety. The development of reference methods and standards for nutrients, contaminants, and adulterants in foods is essential in this regard. Project components include measurement and standards issues related to nutrients in food products, contaminants and adulterants in food products, chemical composition and contaminants in herbal supplements/nutraceuticals, and detection of genetic modifications in food products.

Project priorities are determined in consultation with the AOAC, FDA, , NIH, and the National Food Processors Association.

Environmental Measurements and Standards

Responsible stewardship of the environment is facilitated when measurements of known accuracy are used in monitoring and decision-making. Activities in this project involves the development of reference methods, developing Standard Reference Materials and the provision of other measurement quality assurance services to address measurement problems associated with Drinking Water Quality, wastewater Chlorination/Dechlorination Mechanisms, Atmospheric Monitoring and Global Climate Change Assessment, Automotive Exhaust Emissions, Atmospheric Particulate Characterization, Contaminants in Sediments and Soils, Contaminants in Biological Fluids and Tissues, Specimen Banking Technology.

Activities of this project support directly and indirectly the federal agencies that have stewardship responsibility for the environment (EPA, NOAA, DOE, DOD), state laboratories, and the large, environmental testing service sector that consists of private laboratories, secondary standards producers and proficiency testing firms. Our environmental projects also support industries and their consortia (e.g AIGER, EPRI, etc.) that require high quality and/or traceable measurements to respond in a cost-effective manner to changing regulatory mandates concerning emissions and waste disposal. Our activities also support basic studies and models that track the fate of pollutants over both time and space and their impact on quality of life.

Methods and Standards for Advanced Materials Characterization

Industrial and academic materials scientists often require accurate information regarding the chemical properties of advanced materials. These properties include chemical purity, trace element content, and the distribution of elements within the material. Such information not only allows optimization and control of production processes, but also facilitates a theoretic understanding of both chemical processes and material properties. As the Nation's reference laboratory for chemical measurements, CSTL must not only provide the fundamental basis of the nation's chemical measurement system, but also extend these capabilities to the real world. The Analytical Chemistry Division does this by direct measurements for customers and collaborators, by providing standard reference materials for instrument calibration and for measurement quality assurance, and by making the latest measurement techniques and data available to the advanced materials community.

The Division has a broad array of analytical measurement tools including unique capabilities like neutron activation analysis, prompt gamma activation analysis, neutron depth profiling to provide analytical data and reference measurements needed to provide answers to important measurement problems.

Methods and Standards for Commodities Characterization

The provision of accurate, accepted chemical characterization determinations is critical for those materials whose commercial value, properties, or suitability for use depend on their chemical composition. These characterizations may require measurement of major component(s), identification and quantification of contaminants, and/or determination of the spatial distribution of components. This project requires ongoing identification of those commodity areas with critical needs that can be addressed by NIST and strategic selection of project tasks from among these.

Current project activities focus on sulfur in diesel fuels, composition of metals/metal alloys (e.g., low carbon silicon steel, phosphorized copper), a series of cement SRMs, and an electronic scrap artifact material – the first of a new class of SRMs addressing industrial “green product” goals in

which key decisions, with significant economic and ecological consequences, depend on the quality of analytical characterizations of materials to be recycled.

Microanalytical Technologies – Lab on a Chip

This project is focused on facilitating the design and development of commercial plastic microfluidic systems by developing techniques to achieve greater control of microflow and microchemistries performed in plastic microchannels. In both the analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in microfluidic systems must be well-controlled to provide accurate and reliable results. In response to this need, the goal of our multi-year program is to develop novel methods to evaluate and control chemistries in microscale systems. First, we will develop methods to understand and manipulate microflow in a predictable manner since a lack of flow control can lead to misinterpretation of analytical results. We will also develop methods to accurately evaluate and control temperature profiles in microchannels since temperature is a critical parameter that has a profound effect on reaction kinetics. Finally, we will develop methods to fabricate and integrate components that allow for finer control of chemical reactions including passive micromixers and microarrays. Based on input from a young microfluidics industry, the fundamental control of chemical reactions performed in microchannels is the underpinning of a program designed to promote the commercial realization of plastic microfluidic systems.

Further discussions regarding the Division's core competencies, focused project areas, and products/services delivered to customers are provided in the Groups Section of this Overview and the Selected Technical Activity Reports.

III. SRMs: Tools for Providing Chemical Measurement Traceability

Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating "traceability-to-NIST" and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions. Standard Reference Materials (SRMs) are certified reference materials (CRMs) issued under the National Institute of Standards and Technology trademark that are well-characterized using state-of-the-art measurement methods and/or technologies for chemical composition and/or physical properties. Traditionally, SRMs have been the primary tools that NIST provides to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST catalogs nearly 1300 different types of SRMs; in 2001 NIST sold over 32,000 SRM units to more than 6,500 unique customers. Approximately 16,000 of the units sold were from the ~700 different types of materials that are certified for chemical composition.

During the past year, measurements were made on ~140 SRMs. Fifteen high-priority renewals were completed along with twelve completely new SRMs. Other measurements were made for stability assessment and/or to address customer questions. In order to address questions from the international community concerning the quality of data provided on SRM certificates, NIST 260-136 "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements" was developed. Completed in January, 2000. In addition, a Division Quality Manual that summarizes and formalizes the Division's policies and approaches to

addressing quality-related issues concerning the services that we provide was also completed and placed on the Division's internal website.

Since it has the world's leading, most mature, and most comprehensive reference materials program, most of the world looks to NIST as the de facto source for high quality CRMs to support chemical measurements. NIST has met the reference materials needs of U.S. industry and commerce for nearly 100 years. While our reference materials program has focused primarily on U.S. requirements, it is clear that these materials address international measurement needs as well. As demonstration of quality and "traceability" for chemical measurements have become increasingly global issues, the need for internationally recognized and accepted CRMs has increased correspondingly. Their use is now often mandated in measurement/quality protocols for analytical testing laboratories. Coupled with the fast pace of technological change and greater measurement needs, the demand for additional quantities and additional specific varieties of reference materials has mushroomed. NIST, by itself, does not have the resources to provide SRMs (exact sample types, unique compound combinations, concentrations, etc.) to meet all these needs. Without a significant shift in paradigm, we will not be able to address future needs for reference materials; neither nationally nor internationally.

IV. NTRM Programs for Leveraging NIST Resources and Efforts

The NIST Traceable Reference Materials (NTRM) program was created to partially address this problem of increasing needs for reference materials with a well-defined linkage to national standards. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept was implemented initially in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful. Since its inception, 11 specialty gas companies have worked with NIST to certify over 8500 NTRM cylinders of gas mixtures that have been used to produce more than 500,000 NIST-traceable gas standards, with a market value of approximately \$110M, for end-users. .

Operationally, in the Gas NTRM Program, candidate gas mixtures are prepared in batches of ten or more cylinders, analyzed, and data submitted to NIST. NIST selects 10% of the cylinders in the batch and analyzes them so that NIST can value assign the batch. The cylinders are returned to the SGC with Certification documentation for the batch. The cylinders are under control of the SGC and can be sold to end users to provide a NIST traceable gas or the NTRM mixtures can be used by the SGC to analyze other mixtures that are then sold to provide traceability. The qualifier on the part of NIST is that NIST must have the primary standards available to perform the NIST analyses. Thirty-six NTRMs were value-assigned for four Specialty gas Companies during FY01. According to Stephen Miller, Technical Director, Scott Specialty Gases, "the NTRM program has served as an excellent vehicle for production of the high quality standards - of known pedigree - required by both industry and the regulatory community in the implementation of Title IV [SO₂ emissions trading] of the 1990 Clean Air Act."

The NTRM concept has been expanded to our Optical Filter Standards Program. Currently, this program is just getting underway with three vendors. In the Optical Filters NTRM program, each vendor is certified by the National Voluntary Laboratory Accreditation Program (NVLAP) to produce visible filter standards. Traceability is provided by the use of NIST SRM optical filters to

calibrate vendor spectrophotometers, and quality assurance is afforded by NIST review of all vendor calibration, certification, and recertification data, NIST measurements (one set from each vendor production batch), and blind spot checks. In addition to NTRMs, 176 sets (for 125 different companies) of optical filter standards were re-value assigned for wavelength and/or absorbance in the UV and NIR spectral region 12 during FY01.

We had previously planned to also develop NTRMs in the Elemental Standards area. Those plans are being re-evaluated. Our single element spectrometric solution standards program (SRM 3100 series) is now on its firmest footing since its inception. All elements are now in stock and the uncertainty model was updated to bring the certificates into full ISO GUM compliance. This uncertainty analysis includes incorporation of the new "Type B on Bias" method developed by the Statistical Engineering Division, as well as a different treatment for transpiration correction. This year the certification projects for 7 elements were completed (Th, Er, Pt, Co, Ni, and Ca) and re-certifications for 6 additional elements initiated (Al, As, Cr, Cu, Pb, and Na), all of which anticipate replacement of in-stock materials. The certification of two very difficult elements, rhodium and rhenium, which have been out-of-stock for several years was also completed. Both projects involved considerable research before reliable results were achieved. Stability tests were also completed for five elements (Mn, P, Cs, Ho, Y). This testing was done to evaluate the extended shelf life of ampuled materials. The Plans for the coming year call for the initiation of a new class of elemental standards, NIST Primary Standards (NPS); these materials will be certified for amount of material, as opposed to concentration.

We recognize that the NTRM model is only applicable in selected areas. In the remaining areas, such as complex matrix standards, we have begun to leverage our resources through increased strategic collaborations with other National Metrology Institutes and selected U.S. laboratories. Additional details concerning our collaborative activities with both private sector U.S. laboratories and commercial reference material and proficiency testing service providers as well as other National Metrology Institutes worldwide are provided in Technical Activity Reports that follow.

V. International Standards Activities

International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade and environmental and health-related decision making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. In October, 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention signed the mutual recognition arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). This MRA provides a framework for assessing the degree of equivalence between the measurement capabilities and standards issued by NMIs or NMI-designated labs throughout the world. NIST has taken a leadership role the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM) and the Chemical Metrology Working Group of the Interamerican System for Metrology (SIM) in order to assure the effective, fair and metrologically sound implementation of this MRA.

The CCQM has formed seven working groups: (1) gas analysis, (2) organic analysis, (3) inorganic analysis, (4) electrochemistry (5) Biometrology, (6) Surface Analysis and (7) Key Comparisons.

These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. The Analytical Chemistry Division is currently leading various activities within all five of the seven working groups. During FY01, we participated in 25 CCQM comparison studies; serving as Pilot Laboratory in 13 of those. A summary report of results from CCQM K-6 that has been recently "Approved for Equivalence" is provided in the Technical Activities Reports section as Report # _____. A similar report is required for each key comparison upon its completion.

Participation in CCQM comparisons is available only to top-tier NMIs around the world. Within the Americas, only the U.S., Canada and Mexico have well-established programs in chemical metrology. In order to most effectively address the unique needs of the 34 countries within SIM whose capabilities in chemical metrology span a very broad range, we have initially focused the SIM program on training and capability assessment rather than participation in MRA-driven Key and Supplemental Comparisons. During the past year, six intercomparison exercises were developed to assess the proficiency of SIM NMI's and/or their designated collaborators for addressing chemical measurement problems within their regions and the Americas. While SIM is focussing entirely on training and capability assessment, Regional Chemical Metrology Working Groups in Europe and the Asian Pacific are forging ahead and conducting MRA-driven Key Comparison Studies. We have established agreements with the Chemical Metrology Working Group Leaders of both regions to allow non-CCQM member countries within SIM to participate in such studies as soon as they feel competent to do so.

We are also working with CITAC (Cooperation in International Traceability in Analytical Chemistry) to establish practical, yet metrologically sound, vertical traceability links between the NMIs and chemical testing labs in the various countries and regions around the world.

In addition to these global and regional activities, we are also establishing a limited number of strategic bilateral collaborations and intercomparisons with NMIs around the world. For example, our collaboration with the Netherlands Measurement Institute (NMI) for determining the equivalence of primary gas standards has resulted in a formal "Declaration of Equivalence" that is recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence between seven NIST and NMI primary gas mixture suites. Our formal agreement with NRC-Canada (via the NAFTA Treaty) for cooperation in marine environmental studies has fostered collaborations in the certification of a several certified reference materials important for trade and environmental decision making between the U.S. and Canada. We have recently signed a Cooperative Arrangement with NIMC (Japan) for collaborative efforts in the area of pure volatile organic compound standards. These highly pure reference compounds will serve as primary references for many of our gas mixture SRMs and our quantitative reference database (SRD-79). Several additional strategic bilateral arrangements with other National Metrology Institutes /Standards laboratories are being discussed.

VI. Collaborations with Other Government Agencies and Professional Organizations

Providing chemical measurement quality assurance services in support of other Federal and State government agency programs (on a cost reimbursable basis) continues to be an important part of our measurement service delivery portfolio. During the past year, we were involved with 25 projects with eleven federal and state government agencies. We also had technical interactions that involve laboratory research and measurement activities with more than 20 professional organizations and societies including the American Industry/ Government Emissions Research consortium (AIGER),

American Association for Clinical Chemistry (AACC), American Society for Testing and Materials (ASTM), Certified Reference materials Manufacturers (CRMMA), Association National Food Processors Association (NFPA), National Council on Clinical Chemistry (NCCLS), and the National Environmental Laboratory Accreditation Council (NELAC) Specific details concerning many of these interactions are provided in the Groups Section of this Overview.

VII. Future Directions

We will continue our strategy of maintaining core competencies in chemical measurement science to address measurement problems in areas of national priority. All Division research and service projects will continue to be reviewed on an annual basis for match to mission, progress, quality and customer need.

Several new research activities will be initiated in the coming year. In the **Forensics Standards** area, we will be developing a collaborative program with CDC for providing quality assurance and measurement proficiency assessment for the “Chemical Counter-Terrorism Laboratory Network” which will initially be comprised of several state public health laboratories and the CDC. In the event of a chemical terrorism attack, samples (urine or blood) would be shipped to CDC for analysis to determine what agents were used, who was exposed, and how much exposure occurred. CDC would most likely not be able itself to handle so many samples in a short time that would need the help of the state labs in the analyses. NIST’s responsibility would be to work with CDC and the State Labs to assure the quality of results from such tests. Emphasis of our **Food and Nutrition Measurements** area will be shifted from population of the AOAC Food Triangle to address nutritional labeling issues to quality assurance for Nutraceutical products and detecting of genetic modifications in foods. Research will also begin in a new Competence area, **Single Molecule Measurements and Manipulations**. Additional details concerning research activities in these areas are provided in the Group Sections of this Overview that follow later.

Healthcare is a major focus area for NIST, the Chemical Science and Technology Laboratory and the Analytical Chemistry Division. In addition to the clinical measurement reliability and cost issues that have driven our measurement and standards for clinical diagnostic markers project over the past twenty years, a very significant commerce and competitiveness issue has recently emerged -- the European Directive 98/79/EC on *in vitro* diagnostic medical devices. By December 2003, manufacturers must declare that any new IVD product to be sold within the EU complies with all “essential requirements” of this Directive. One of these requirements is that IVD products be traceable to “standards of the highest order”, e.g., nationally and/or internationally recognized reference methods and/or certified reference materials. At present, IVD devices are used in clinical laboratories to measure more than 300 different chemical or biochemical species. Reference methods and/or materials exist for about 30. Approximately 60% of the IVD products currently on the European market are imported from the USA. Excluding home diagnostics, the overall world wide in-vitro diagnostic market ("IVD") is approximately \$20 billion.

In response to this need we have committed to develop new reference methods and blood/urine-based SRMs for several new health-status markers while maintaining previously developed reference systems for calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium,

potassium, sodium, triglycerides, urea , uric acid, and vitamins A,C, E and beta carotene. Over the next two years, reference methods and standards will be developed for:

- **Bilirubin** (*liver function*)
- **Cardiac Troponin I** (*heart attack occurrence and damage*)
- **Cadmium** (*toxicity*)
- **Cortisol** (*endocrine function*)
- **Folates** (*neural tube defects*)
- **Prostate Specific Antigen** (*prostate*)
- **Glycated Hemoglobin** (*diabetes status*)
- **Homocysteine** (*heart attack risk*)
- **Ionized Calcium** (*important marker for diagnosis of various disease states such as skeletal resorption and stone formation in the urinary tract*)
- **Mercury** (*toxicity*)
- **Speciated Iron** (*anemia and hemochromatosis*)
- **Thyroid Stimulating Hormone, Triiodothyronine and thyroxine** (*thyroid function*)
- **Total and Speciated Selenium** (*to support clinical monitoring in the relatively narrow range of beneficial effect*)

Over the next decade, driven by the availability of new sensor-based measurement technologies, more and more clinical testing will be done outside the traditional clinical laboratory. The annual U.S. market alone for this new form of clinical measurements, called point-of-care testing (POCT), is currently a billion dollars and is growing at an annual rate of 10%. POCT is expected to be used extensively in the home as part of a self-care trend, which is currently experiencing a 70% growth rate. Published studies have concluded that POCT provides at least the same level of diagnostic value as centralized testing, but at half the cost. The standards infrastructure that has supported clinical chemistry for the past two decades must adapt to support POCT. Collaborative efforts need to be established among National Standards Laboratories, in-vitro diagnostic device (IVD) manufacturers, and others in the medical professional community to develop appropriate technologies and non-biohazardous standards to facilitate the provision of data used in medical decision-making that are accurate and traceable to national/international standards. NIST leadership in developing traceable POCT standards will help assure continued U.S. dominance of the worldwide IVD market and foster more affordable healthcare both at home and abroad.

The most widely used home testing devices are for glucose. Diabetes affects more than 10 million Americans and its prevalence rose from ~5% to 7% during the 1990's. Although home blood glucose monitoring has clearly revolutionized diabetic care and changed both the therapy and outcome for this disease, use of these testing systems remains problematic. In the short term, we will CDC and FDA in their investigations of the performance of home glucose monitors. We have been asked to provide the reference method and whole blood reference materials to support these studies. In the longer term, we will be developing optical artifact standards for non-invasive measurement technologies.

While focusing increased attention on the health-care/clinical measurements area over the next 3-5 years, we will still address critical measurements and standards needs in other areas. The following SRMs are scheduled for completion during the coming year:

- **Anode Tin** (to support lead and material purity measurements for this important steel coating material manufactured, used, and recycled worldwide; lead content of the recycled high-value tin is of particular importance for food safety, product liability, and environmental concerns)
- **Baking Chocolate** (to complement suite of SRMs developed in response to new nutritional labeling laws)
- **Drinking/Wastewater Standards** (to provide accuracy benchmark and measurement traceability for proficiency testing of >5000 environmental laboratories conducted by commercial providers) additional SRMs added to suite such as additional VOCs in methanol, renewals of needed SRMs such as pH, electrolytic conductivity, bromide and fluoride anion solutions, and selected elemental solution standards; Reissue of SRM 1643, Trace elements in Water, as testing laboratory benchmark
- **Fluorescein Fluorescence Intensity** (in collaboration with the Biotechnology Division for calibration of instrumentation widely used by clinical and biotechnology communities)
- **Gravimetric Elemental Primary Standards** (“Mole standards for metals”)
- **Gunpowder Composition** (to validate forensic methods used to identify gunpowder residues)
- **Low Sulfur and Mercury Coal** (to support lower EPA emissions regulations for sulfur and mercury)
- **NIR Transflectance/Reflectance** (wavelength standards suitable in form for calibration of single- and double-pass transmission in both wavenumber and wavelength)
- **Toxic Elements in Freeze Dried Urine** (to provide QA for toxic metal exposure measurements)
- **Phosphorus Implant in Silicon Depth Profile Standard** (with Div. Surface and Microanalysis Science Division, to complement recently issued boron and arsenic implant SRMs for critical calibration of SI mass spectrometers in support of the semiconductor industry)
- **Raman Spectroscopy Standard** (in collaboration with the Surface and Microanalysis Science and Process Measurements Divisions as second in suite of SRMs to calibrate Raman instruments used in pharmaceutical and agricultural industries and other process control applications)
- **TiAl Alloy for Microanalysis and XRF** (with the the Surface and Microanalysis Science Division to provide benchmark for aerospace industry for Ti, Al, Nb, W in USAF-supplied alloy material)

Projects that are in progress with expected completion dates in FY 2003 include:

- **Additives in Smokeless Gunpowder** (for 3 propellant additives; to provide accuracy benchmark and support QA and proficiency testing for explosives-type measurements of state, local and federal forensic laboratories and US military propellant laboratories)
- **Low Nitric Oxide Standards** (to support measurements needed for development of very low emission vehicles and future automobile emissions regulations)
- **Peanut Butter**
and
- **Spinach** (to complete population of AOAC Food Triangle with SRMs for quality assuring measurements made to monitor nutritional labeling compliance)

We expect that the NTRM approach will continue to expand as the basis for allowing the commercial sector to provide reference materials to end-users with a well-defined traceability linkage to NIST. High quality SRMs will continue to be developed to address both national and international measurement problems, but they will be much more expensive and targeted for purchase primarily by other national metrology institutes and commercial producers/distributors.

Increasingly, end-user needs will be met via NIST-traceable commercially produced reference materials.

The complex nature of chemical measurements coupled with the increasingly global nature of trade, health, and environmental issues speaks to the need for having the world make measurements using a common “meterstick”. The comprehensive nature of the NIST program in chemical measurements puts us in position to make a very significant contribution to the international chemical measurements community in this regard. In order to do this in both a cost-effective and internationally congenial manner, we are investigating opportunities for partnering with other highly qualified NMIs to provide high quality Certified Reference Materials in specific standards areas. Potential advantages of these proposed partnerships include increased supply, breadth and quality of reference materials for the worldwide chemical measurements community as well as less duplication of effort for more efficient use of limited resources.

This overview plus the Group-specific sections and Selected Technical Activity Reports that follow provide additional details concerning our work and reflect the high quality of our staff. This information also shows how our efforts are impacting U.S. industry's productivity and competitiveness as well as providing the measurement and standards infrastructural support for environmental quality and human health assessments. They also illustrate the critical synergistic relationships that exist among our research programs in chemical measurement science and the standards and quality assurance services that we provide to customers.

VIII. Group Overviews

Spectrochemical Methods

Research activities in the Spectrochemical Methods Group are directed toward the development, critical evaluation, and application of techniques for the identification and measurement of inorganic species using x-ray, optical, and mass spectrometries. The focus of this research is measurement accuracy and precision, benchmarking industry's needs for traceability, advanced materials characterization, and commodity value assignment and specification.

Development of a new Reference Method for mercury (Hg) was one highpoint of this past year's activities. The cold vapor isotope dilution inductively coupled plasma mass spectrometric method of measurement (CV-ID-ICP-MS) developed last year was refined and applied to a wide range of materials—commodities, as well as clinical and environmental matrices. Research and Development Magazine recognized this measurement technology as among the 100 most innovative in the United States in its presentation of a 2001 R&D 100 award to its inventors W. Robert Kelly and Stephen E. Long.

The emission of mercury from coal-fired power plants is one of the most important regulatory issues for the electric power industry and prior to this year there were no NIST coal SRMs with mercury certified. Now, all seven of the NIST SRM coals have been certified for their mercury content. Because of its responsiveness to a major industry need, this work was nominated by the ACD for the FY 2001 CSTL Technical Achievement Award. In addition to the coals, the mercury content of crude and refined oils was surveyed. Although coal burning is believed to be the major anthropogenic source of mercury emissions, the refinement and combustion of liquid fossil fuels is a potential source for which sparse data exist. The NIST measurements will be extremely useful in

this source term evaluation. Further information concerning our work in this area can be found in Technical Activities # _____.

Mercury is just one of a number of toxic elements for which environmental exposure is a worrisome health issue. In the last decade the major toxic element of concern was lead (Pb) and we have completed an extensive program of standards development and certification to benchmark accurate measurement of lead in environmental and clinical samples. Last year, certification of SRM 966 Toxic Metals in Bovine Blood was completed, which in addition to lead had mercury and cadmium certified. This material complements the previously (1998) certified SRM 955b Lead in Blood.

We are currently engaged in certifying the normal and elevated levels of analytes in a freeze-dried urine renewal material SRM 2670a. This SRM will expand the suite of toxic elements certified in a clinical matrix further—to elements such as arsenic, chromium, thallium, uranium, and thorium—in addition to lead, mercury, and cadmium. As the kidneys regulate elemental metabolism directly through urinary excretion, the concentration of toxic elements in urine has been recognized for some time as one of the most efficient means to biomonitor exposure. Some of the toxic elements of concern—such as arsenic, uranium and thorium—are naturally found at elevated concentrations in certain areas of the United States, such as the American Southwest. In addition, many of these elements have important roles in industrial processes and materials. In contrast to the original urine SRM (SRM 2670) where some of the elements were unrealistically high and exceeded clinically and biologically significant action levels, the levels of the toxic elements in SRM 2670a have been carefully chosen to help the clinical community accurately benchmark biological indices of exposure. This work is being done in collaboration with the CDC, and the SRM should help to benchmark the many measurements that CDC is making through its NHANES effort (<http://www.cdc.gov/od/oc/media/pressrel/r010321.htm>).

In contrast to toxic elements, iodine is an essential micro-nutrient that is used by the thyroid gland to synthesize the hormones thyroxine and triiodothyronine. Iodine deficiency causes an increased risk for metabolic disorders, goiter, and, in the case of infants, impaired neurological development. This risk is largely preventable by administration of iodine in consumed foodstuffs. Almost all of the iodine excreted in urine is present as iodide and this represents a good health marker for iodine loading and intake. The World Health Organization (WHO) has provided guidelines on iodine status as a function of urinary iodide levels, but this clinical measurement has not been supported by the availability of accurately certified reference materials. This year a new analytical method employing isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) was developed. The method utilizes the addition of a long-lived radioactive isotope (^{129}I), which can be calibrated in the usual manner with a stable iodine primary standard. SRM 2670a Toxic Elements in Freeze-Dried Urine has also been analyzed for its iodine content. Iodine certification in this SRM will provide a traceability basis for clinical laboratory measurements of urinary iodine. Future measurements of iodine in human blood serum are also planned.

Iron is also an essential element for life and serum iron determination is an important clinical measurement. Low serum iron levels are indicative of diseases such as anemia, rheumatoid arthritis, and certain infections. High serum iron levels are also indicative of various diseases, including hemochromatosis, an inherited disorder that causes the body to absorb and store too much iron, often resulting in failure of the liver, heart, or pancreas. This year the Group collaborated with the Organic Analytical Methods Group to develop a reference method for speciated iron. Typically, for the purpose of medical diagnosis, the total serum iron concentration is measured. However, in many iron-related diseases, the distribution of iron bound to serum proteins is altered. For

improved medical diagnosis, it would be beneficial to determine the distribution of iron among serum proteins as well as the total serum iron concentration.

The two important iron-containing proteins in serum are transferrin and ferritin. Transferrin is the most abundant iron-containing protein, and most clinical measurements involve transferrin determinations. The concentration of ferritin in serum is approximately one thousand times lower than that of transferrin. However, medical research is indicating that serum ferritin determination can often be a more selective diagnosis tool than determination using transferrin. Development of a quantitative method for serum transferrin using liquid chromatography coupled with mass spectrometry (LC/MS) and affinity chromatography is underway. To provide the information on the concentration of iron associated with the transferrin, we are using high-resolution inductively coupled plasma mass spectrometry (ICP-MS). High resolution is required because ^{56}Fe suffers a major interference from the $^{40}\text{Ar}^{16}\text{O}$ species produced in the Ar ICP. Together, the LC/MS and the ICP/MS measurements provide highly selective and high precision values for both serum transferrin and serum iron levels. The goal will be to produce a reference material for speciated iron in serum that will meet the needs of the majority of clinical iron measurements performed.

We continue to underpin the National Reference System for the Clinical Laboratory (NRSCL) through our development and application of mass spectrometric methods for isotope dilution analysis of electrolytes (calcium, chloride, lithium, magnesium, and potassium). In 2001, an isotope dilution procedure for the determination of potassium and calcium using ICP-MS in the cold plasma operational mode was explored. Thermal ionization mass spectrometry (TIMS) has traditionally provided the accuracy base for the determination of potassium and calcium, but ICP-MS is simpler and more widely available. Ratio measurement precision of ICP-MS can be comparable to TIMS when competently applied. The measurement of K and Ca isotopes by ICP-MS is hindered by the high background from the Ar plasma. However, low power or "cold plasma" conditions can significantly reduce Ar-based interferences, and commercial ICP-MS instruments can be configured to operate in this mode. The increased tendency to form polyatomic ions from the matrix in this mode of operation can affect accuracy, but cation exchange chromatography was applied to completely remove matrix-induced interferences, thus allowing the determination. (Separations are also required by TIMS, for formation of a stable ion beam). The determination of Ca in serum was demonstrated and the results benchmarked against TIMS.

Calcium was determined in a frozen serum material as part of the CCQM Key Comparison P-14. SRM 956a Electrolytes in Frozen Serum was analyzed as a control. Isotope ratios were determined using both ICP-MS and TIMS. The ICP-MS/TIMS ratio of concentrations determined was 0.9999 ± 0.0016 (1s, N=5), illustrating the good comparability between the two techniques. The result for SRM 956a was +0.28 % relative to the certified value, which was well within the relative certified uncertainty of ± 0.63 %. These results again validate the inherent accuracy and stability of a measurement system based on IDMS.

One of the major occupational health issues for the Nation's construction and mining industries is silicosis. Nearly 2 million U.S. workers (and millions more worldwide) are potentially exposed to crystalline silica, which causes this debilitating and incurable, but entirely preventable lung disease. Silica is difficult to measure accurately, as demonstrated by the poor agreement in interlaboratory comparisons. One of the main reasons is that a suitable reference material for silica is unavailable. Consequently, private and public resources are diverted from correcting silica exposure problems to contesting enforcement cases. Thus, the Occupational Safety Health Administration (OSHA), National Institute for Occupational Safety and Health (NIOSH, a branch of CDC) and the Mine

Safety Health Administration (MSHA) and industry representatives are working with NIST to develop a Standard Reference Material that is appropriate for silica calibration.

The creation of a stable silica-on-filter reference material has been investigated and the feasibility for commercial production explored. A certification plan for the reference material has been established and methods developed and validated for sample digestion and silicon determination. Silicon on prototype filters has been determined using both inductively coupled plasma optical emission spectrometry and high resolution ICP-MS with comparable results. The production and certification of the SRM is slated for the coming year.

Interface characterization and the analysis of thin layers and coatings is an important analytical problem in a wide variety of modern technologies. The need to develop better, faster, and more economical characterization methods to determine elemental composition quantitatively as a function of depth will surely grow. We have investigated glow discharge optical emission spectrometry (GDOES) for this purpose this year, with support from the CSTL Exploratory Research Program, and in collaboration with the Surface and Microanalysis Science Division. The initial research focused on the conditions that affect uniform sputtering during the glow discharge process. SRM 2135c Nickel Chromium Depth Profiling standard has been the principal test sample. This Ni/Cr multilayer stack has been thoroughly characterized using secondary ion mass spectrometry (SIMS) and Auger emission spectrometry. Encouraging depth profiles have been achieved (reported in an accompanying Technical Activity Report).

The glow discharge sputtering process was shown to depend on the both the physical and chemical nature of the sample. Samples of brass, monocrystalline silicon, and electrolytic nickel were sputtered at various sets of discharge conditions, and the resulting craters were evaluated. Even in the single crystal Si, a flat crater was not formed—the edges sputtered faster than the center. Similar types of sputtering effects have been studied extensively in SIMS but have not been documented in GDOES. It is obvious that quantitative depth analysis will require better physical understanding and control of this sampling process.

The X-Ray Fluorescence (XRF) team continued to refine the development of a matrix-independent method based on borate fusion (reported in an accompanying Technical Activity Report). This method provides direct traceability to primary reference materials in combination with the speed and repeatability of XRF. The method has been applied to the certification of cements and zeolites. This year, its applicability to the analysis of metals and metal alloys was studied in depth. A direct comparison was made between calibrations from disk-form low alloy steels and fused, chip-form low alloy steels. Calibrations for 20 elements were created using a straight-line model for both fused and disk forms. The performance of bracket standard calibration for low alloy steel demonstrated matrix-independence with traceability to primary reference materials. The application of this calibration method was applied to the analysis of SRM 361 and resulted in excellent performance even for trace As, normally considered a volatile element. In general, this work demonstrates that XRF will no longer be limited to disk-form specimens, which is especially important for alloys that do not form homogeneous solids. In fact, the superior speed and repeatability of XRF can be used to great advantage for analysis of metals, alloys, ores and related materials with fewer certified reference materials.

The identification and measurement of elemental species is a prominent topic in many areas of science. This topic was central to a workshop at the most recent BERM meeting, and discussions have been held with EPA and other agencies about the need for environmental standards certified

for species (e.g. arsenic, chromium, and mercury). As discussed above, we have initiated research for clinical iron species. This year we have also initiated a collaborative research effort in species measurement with Professor Christine Grant and her students (North Carolina State University). The use of calcium ion sequestrants as environmentally benign alternatives to strong acids for the cleaning of mineral deposits, typically calcium phosphate, calcium oxalate and calcium carbonate, on stainless steel surfaces is being explored. The research requires the identification and quantification of calcium and its species on-line under various mechanical cleaning conditions. This summer, a tandem technique was developed that incorporated size-exclusion chromatographic (SEC) separation of calcium species, spectrophotometry and inductively coupled plasma optical emission spectrometric (ICPOES) detection techniques. This problem, the formation and removal of mineral deposits (scale), is important for many industries.

The single element spectrometric solution standards program (SRM 3100 series) is now on its firmest footing, probably since its inception. All elements are now in stock and the uncertainty model was updated to bring the certificates into full ISO GUM compliance. This uncertainty analysis includes incorporation of the new “Type B on Bias” method developed by the Statistical Engineering Division, as well as a different treatment for transpiration correction. This year the certification projects for 7 elements were completed (Th, Er, Pt, Co, Ni, and Ca) and re-certifications for 6 additional elements initiated (Al, As, Cr, Cu, Pb, and Na), all of which anticipate replacement of in-stock materials. The certification of two very difficult elements, rhodium and rhenium, which have been out-of-stock for several years was also completed. Both projects involved considerable research before reliable results were achieved. Stability tests were also completed for five elements (Mn, P, Cs, Ho, Y). This testing was done to evaluate the extended shelf life of ampuled materials. The Plans for the coming year call for the initiation of a new class of elemental standards, NIST Primary Standards (NPS); these materials will be certified for amount of material, as opposed to concentration.

The basis of the single element spectrometric solution standards’ certification is high performance ICP-OES. The underpinning science in the application of this technique to the program was documented this year in Analytical Chemistry [Marc L. Salit, Gregory C. Turk, Abigail P. Lindstrom, Therese A. Butler, Charles M. Beck II, and Bruce Norman ; “Single-Element Solution Comparisons with a High-Performance Inductively Coupled Plasma Optical Emission Spectrometric Method,” Analytical Chemistry; 2001; 73(20); 4821-4829]. An alternative mechanism for technology transfer of HP-ICP-OES has also been pursued this year through the development of a software tool that guides an analyst through the measurement process. This tool was beta-tested with two commercial standards companies that wish to use HP-ICP-OES to perform traceability comparison measurements between their products and the SRM 3100 standards. The results are encouraging. With this capability, they can now value-assign their standards based on our SRMs with uncertainties that are nearly identical to the SRM uncertainties.

The Group participated in four pilot and key comparisons of the inorganic working group of the CCQM this year. CCQM K-13 key comparison of cadmium and lead amount content in sediment was a successor to the CCQM P-15 study (NIST did not participate). Satisfactory agreement of reported results among the NMIs was observed in K-13, although several laboratories did not adequately account for a Sn interference during Cd measurement—this was not a problem for the pilot material which had much lower Sn content. The median value was chosen as the key comparison reference value (KCRV) with the exclusion of two NMI results. NIST results were both within 0.24 % of the KCRV. NIST and NRC-Canada results agreed within 0.61 % for Cd and were

the same for Pb. All of these results—NIST, NRC-Canada, and the KCRV—were indistinguishable within stated uncertainties.

CCQM P-12 was focused on measurement of the Pb content in wine. Fifteen NMIs reported results, which were in excellent agreement at the 25 ng/g level. NIST results were within 0.01 % of the median value. Twelve of the 15 laboratories agreed within 2 % of the median value. The consensus of the working group was that this comparison should have been instituted as a key comparison, not a pilot comparison.

CCQM P-26 Sulfur in Fuel was completed in June; the results have not yet been presented. This pilot comparison was sponsored by IRMM, using two materials that will be certified as BCR (European Community Bureau of Reference) CRMs. NIST and IRMM collaborated on the certification (in progress) of these CRMs (and a third material not distributed in the CCQM comparison). Previous BCR sulfur in fuel CRMs were certified by European interlaboratory exercises. For this certification, it was decided to deliver results directly traceable to the SI using “primary methods of measurement.” Thus, the characterization measurements performed by NIST and IRMM were both the isotope dilution thermal ionization mass spectrometric (ID-TIMS) method developed at NIST. This work was the first application of ID-TIMS by IRMM and they made a number of changes in their adoption of the method, including microwave dissolution instead of the Carius tube technique used at NIST. The results were not in as good agreement as we would have expected; there was a relative bias between 1 to 2 % between the two laboratories. This experiment and intercomparison gives us the opportunity to re-evaluate the primary nature of the ID-TIMS method.

CCQM P-14 Calcium in Serum was completed in July; the results have also not been presented. The measurements at NIST were made by both ID-TIMS and ID-ICP-MS as described above and benchmarked against SRM 956a Electrolytes in Frozen Serum. The precision and accuracy of the results provide good confidence in our capability for application of this definitive method.

The CCQM inorganic working group has one pilot comparison ongoing (CCQM-P29 Cadmium in Rice) and will be initiating further work at its first meeting in October 2001. The results for the completed pilot comparisons discussed above could lead to several new key comparisons in the coming year. These CCQM and other international interactions (CITAC, SIM)—comparative measurement programs, traceability and uncertainty discussions, and measurement capability assessments and claims—promote the development of a global infrastructure for chemical measurement, an infrastructure where NIST must represent the highest U.S. interest.

The above summary illustrates the wide range of research and measurement activities studied by the Group in the past year, using the spectrometric techniques available. The Group also made significant contributions to Division Standard Reference Material projects not mentioned above; inorganic constituents were determined in: Pine Needles, Inorganic Sediment, Mine Waste, Trace Elements in Water, Baking Chocolate, Peanut Butter, KDP, Steels, Anode Tin, Copper Alloys, Crude Oil, Coals, and more

Organic Analytical Methods

Research activities in organic analytical methods are directed toward the development, critical evaluation, and application of methods for the identification and measurement of organic and organometal species using mass spectrometry and analytical separations techniques. These separation techniques include gas chromatography (GC), liquid chromatography (LC), supercritical

fluid chromatography (SFC) and extraction (SFE), capillary electrophoresis (CE), and capillary electrochromatography (CEC).

Recent research activities in organic mass spectrometry have focused on the development of techniques for characterization and quantitative determination of proteins in biological matrices. Levels of specific proteins and other biomolecules in blood are indicative of certain disease states. These so called "health status markers" often permit more rapid diagnosis of disease with greater certainty than is possible by other methods. Twelve health status markers have been identified as highest priority for immediate studies. This year efforts have been directed toward the development of reference methods for troponin I (a new marker of myocardial infarction), thyroxine (a marker for thyroid function), cortisol (a marker for endocrine function), speciated iron (how iron is associated with proteins is important for elevated or low iron levels), homocysteine (a risk factor for myocardial infarction), folic acid (an essential nutrient that reduces the risks of heart disease and neural tube defects), and prostate specific antigen (a marker for prostate cancer). Because protein biomarkers occur naturally with considerable heterogeneity (i.e., with glycosylation, acetylation, phosphorylation), we have developed techniques for chemical characterization of the target species. For example, techniques have been developed for the characterization of purified troponin I preparations, thus providing a means for comparing the molecular structure with results from field studies involving immunoassays. An important aspect of the health status marker program is commutability. To be useful to the clinical community, biomarker levels in reference materials certified by primary and/or reference methods must be commutable to field methods. To this end, an interlaboratory comparison exercise of candidate troponin I reference materials was carried out in collaboration with the Troponin Subcommittee of the American Association of Clinical Chemists. Ten troponin I materials were evaluated by 13 manufacturers of immunoassays. A second interlaboratory comparison with the best candidate materials from the first study is being organized. The most suitable troponin I preparation from this second study will be identified for issuance as an SRM in the coming year.

Acquisition of an LC/MS/MS instrument during the past year increased our capabilities for the determination of analytes of health, nutritional, forensic, and environmental importance, as well as for structural studies of natural products. Another new instrument, a matrix assisted laser desorption time-of-flight mass spectrometer (MALDI-TOF) system, was obtained to further enhance our capabilities for characterizing biomolecules. This instrument was acquired with support from the Defense Threat Reduction Agency to begin development of protocols for generation of mass spectra from bacteria. The combination of proteins produced by one species of bacteria differs from that of another. Even different strains of the same species can generate significantly different combinations of proteins. The MALDI-TOF is used to generate a mass spectrum from the bacterial proteins. However, the patterns are affected by how the bacteria are treated and by the matrix compounds added to facilitate the MALDI-TOF process. Once protocols are established and agreed upon by researchers in this field, a searchable database will be created. Such a system will permit the identification of bacteria much faster than is now done by conventional approaches and will be applicable to counter terrorism activities as well as to other applications such as food safety. The MALDI-TOF system will also be used in characterizing health status protein markers as well as proteins expressed by genetic modification of foods.

Research activities in separation science continue to focus on investigations of the physical and chemical processes that influence retention in LC, GC, SFC, CE, and CEC. Results from these fundamental studies are used to design stationary phases tailored to solve specific separation and analysis problems, and to assist in method development and optimization. Recently we have

explored a novel approach to the synthesis of LC stationary phases based on polymer immobilization. Polyethylene acrylic acid copolymers were immobilized on silica as an alternative to conventional silane surface modification chemistry. The resulting columns were evaluated for the LC separation of carotenoid isomers, and preliminary results indicate exceptional selectivity for this class of compounds. Further optimization of this promising stationary phase should result in improved measurement capability for carotenoids and related nutrients in food and tissue samples. Research in chiral separations is continuing in several areas using LC, CE, and GC. The determination of chiral drug species in hair samples using LC may permit environmental exposure to be distinguished from illicit use. Method development is in progress for the determination of amphetamine and methamphetamine enantiomers in hair extracts. In other studies, functionalized cyclodextrins have been evaluated as chiral selectors in CE. Three commercially available sulfated cyclodextrins with similar degrees of sulfation were chosen for study. A capillary electrophoresis method with indirect detection was used to characterize the patterns of sulfate substitution of these materials. The selectors were then investigated as chiral additives in capillary electrophoresis. The electrophoretic results highlighted the importance of both the degree of substitution and the location of sulfate substituents on the cyclodextrin. The strongest analyte-selector interactions were observed for the randomly substituted material. These studies emphasize the importance of the use of well-characterized selectors for reproducible results in chiral CE.

Our research in organometal speciation has continued with improvements in the GC-atomic emission (AED) method for methylmercury and alkyltin species. The new approach involves derivatization and a solid phase microextraction (SPME) step to concentrate the analytes prior to GC-AED analysis. This approach has been used to provide data for certification of methylmercury in SRM 2977 Mussel Tissue, SRM 1566b Oyster Tissue, and SRM 1946 Lake Superior Fish Tissue. This same general approach has also been used to measure butyltin species in several of the sediment-matrix SRMs (i.e., SRM 1941b Organics in Marine Sediment, SRM 1944 New York/New Jersey Waterway Sediment, and SRM 1646a Estuarine Sediment). The butyltin results will be provided as reference values for these SRM.

Other separation science research has involved the development of multidimensional chromatographic approaches for isolation and quantification of nonortho-substituted polychlorinated biphenyl congeners (considered to be the most toxic congeners) in some of our existing SRMs (cod liver oil, sediment, and whale blubber). This method was applied for the certification of these nonortho congeners in SRM 1946 Lake Superior Fish Tissue, which will be the first natural matrix SRM to have certified values for these toxic PCB congeners. We also improved the methodology for the determination of nitro-substituted PAHs in environmental matrices using a normal-phase LC isolation of the nitro-PAHs prior to measurement by GC/MS with negative ion chemical ionization detection. An improved separation of the isomeric nitro-PAHs, particularly the nitropyrene and nitrofluoranthene isomers, was achieved using a 50% phenyl-substituted methylpolysiloxane. This improved methodology was used to assign values for concentrations of mononitro-PAHs and dinitropyrene isomers in three diesel particulate-related SRMs (SRM 1650a, SRM 1975, and SRM 2975) and two air particulate SRMs (SRM 1649a and SRM 1648).

During the past year, measurements have been completed on a number of SRMs of importance to the clinical, environmental, and food and nutritional communities:

- **Clinical Reference Materials**

SRM 913a Uric Acid. SRM 913a is a standard used for calibrating assays for serum uric acid, a measurement used for diagnosis of gout. A variety of analytical techniques were used to detect and measure impurities in this material. These results were evaluated to determine the overall purity and uncertainty of this material.

- **Environmental Reference Materials**

Diesel Particulate SRMs. Three diesel particulate materials were issued during the past year. SRM 1650a Diesel Particulate Matter, first issued in 1985, was reanalyzed and issued with certified/reference values for ~56 PAHs and nitro-PAHs. SRM 2975 Diesel Particulate Matter (Industrial Forklift) is a contemporary diesel particulate material collected from an industrial forklift. SRM 1975 Diesel Particulate Extract is a solvent extract of the particulate material used to prepare SRM 2975. Both SRM 1975 and SRM 2975 were developed at the request of EPA to provide reference materials representative of emissions from modern diesel engines. Certified and reference values are provided for ~50 PAHs and nitro-PAHs. In addition, mutagenicity data provided by EPA is reported for SRM 1975.

SRM 1941b Organics in Marine Sediment. SRM 1941b is the third issue since 1989 of the popular marine sediment SRM for organic contaminants. Results from multiple analytical methods were combined to provide certified values for approximately 25 PAHs, 30 PCB congeners, and 10 chlorinated pesticides. The PAH, PCB, and pesticide concentrations are approximately 2 to 20 times lower than those in SRM 1944 New York/New Jersey Waterway Sediment, thus these two sediment materials will serve the needs of the marine environmental measurement community for both high and moderate levels of organic contaminants.

SRM 1946, Lake Superior Fish Tissue. SRM 1946 is a cryogenically homogenized fish fillet prepared from adult lake trout (*Salvelinus namaycush*). The fish tissue homogenate is indicative of urban contamination levels and will be beneficial to both the environmental and food/nutrition measurement communities. Certified values have been determined for 30 PCB congeners (including 3 non-ortho congeners), 13 chlorinated pesticides, methylmercury, and total mercury. This is the first SRM available with certified concentrations for the non-ortho PCB congeners, which are considered the most toxic, similar in structure to polychlorinated dibenzo-p-dioxins.

SRM 1974b, Organics in Mussel Tissue (*Mytilus edulis*). SRM 1974b is the third issue since 1990 of the mussel tissue SRM for organic contaminants. Certified values will be determined for approximately 40 PAHs, PCBs, and pesticides, as well as methylmercury and total mercury.

Instrument Calibration Standards. Six calibration solution SRMs to support measurements of contaminants in the marine environment were completed last year, including SRM 2269 and SRM 2270 Perdeuterated PAH Solution in Hexane/Toluene, SRM 2273 DDTs and Metabolites in Iso-octane, SRM 2275 Chlorinated Pesticide Solution-II in Iso-octane, SRM 2276 Three Planar PCB Congeners in Iso-octane, and SRM 2274 PCB Congener Solution-II in Iso-octane.

A suite of 26 organic calibration solution SRMs has been developed to support the recently externalized Water Performance Evaluation (PE) Studies Program orchestrated by the US Environmental Protection Agency (USEPA). These SRMs are intended to support the PE program; however, it is anticipated that widespread usage will result after issuance. These SRMs include: six arochlor mixtures in methanol and in oil; chloral hydrate, haloacetic acids, dioxin, endosulfan, chlorinated herbicides, toxaphene, chlordane, and adipate/phthalates in methanol; organochlorine pesticides in acetone; glyphosphate and diquat dibromide in water; and vydate and carbamates in acetonitrile.

- **Food Reference Materials**

The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed. AOAC (Association of Official Analytical Chemists) International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC's belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods. NIST has been working with other government agencies and the food industry over the past several years to provide an increased array of SRMs with values assigned for proximates (procedurally defined values for fat, protein, carbohydrate, etc.), fatty acids, cholesterol, vitamins, elements of nutritional interest, etc. Concentration values in the food-matrix SRMs are assigned based on a combination of measurements from NIST and interlaboratory comparison exercises involving approximately 20 member laboratories of the National Food Processors Association's (NFPA's) Food Industry Analytical Chemists Subcommittee (FIACS).

SRM 2384 Baking Chocolate. SRM 2384 has values assigned for proximates, individual fatty acids, total dietary fiber, several vitamins, catechins, theobromine, caffeine, and elements of nutritional interest.

SRM 1946 Lake Superior Fish Tissue. SRM 1946 is described above as an environmental matrix SRM, but is also useful as a food-matrix. Therefore SRM 1946 also has values assigned for nutrients including proximates (fat, protein, etc.) and individual fatty acids, including several omega-3 fatty acids.

SRM 2385 Spinach and 2387 Peanut Butter. These two SRMs have been prepared and value assignment is in progress for nutrients, carotenoids, and pesticides in the spinach and for nutrients, allergens, aflatoxins, and amino acids in the peanut butter.

- **Botanical SRMs**

The U.S. 1998 market for nutraceuticals (i.e., herbal supplements and functional foods) was estimated at nearly \$26 billion; botanicals accounted for about \$3.5 billion of this market, and a continued growth rate of 10% to 20% was expected. However, in 1998 sales of botanical products began to level off, and by the end of 1999, overall sales had dropped 3% compared to 1998. Part of this decrease in sales may be attributed to a loss in consumer confidence in botanical products as a result of negative publicity. Reports of inaccurate labeling, adulteration, contamination (pesticides, heavy metals, or toxic botanicals), and drug interactions have all contributed to the decline in sales. Taxonomically authentic botanical reference materials with assigned values for active and/or marker

compounds, as well as for contaminants such as pesticides, mycotoxins, and heavy metals, are necessary for verification of manufacturers' label claims and quality control during the manufacturing process.

We have developed a collaborative program with the Food and Drug Administration's (FDA's) Center for Food Safety and the National Institutes of Health's (NIH's) Office of Dietary Supplements to produce a number of authentic, well-characterized reference materials of botanical raw materials to support the needs of the dietary supplement community. Starting in FY02 ACD, FDA, and NIH will collaborate to prepare six to eight selected botanical-matrix SRMs from authentic raw materials during the next several years. FDA, with input from other agencies and organizations, will consult with NIST to identify the materials of high priority.

- **Instrument Performance Standards**

Insights gained from our research in separation mechanisms have provided us the knowledge to develop two new LC column performance test mixture SRMs. SRM 870 Column Performance Test Mixture has been developed to characterize LC column properties such as silica silanol activity, trace metal activity, and separation efficiency. SRM 877 Chiral Selectivity Test Mixture for Liquid Chromatography has been developed to facilitate prediction of the performance of columns for resolving enantiomers.

Gas Metrology and Classical Methods

Research activities within the Gas Metrology and Classical Methods Group are focused on gas metrology, classical wet chemical methods (gravimetry, titrimetry), coulometry, ion chromatography, and maintenance of the theoretical infrastructure for pH and conductivity measurements.

During FY01 the following SRMs were completed; 10 gaseous, 6 conductivity, 3 Volatile Organic Compound solutions and a titanium dioxide material. A total of 104 gas cylinder SRMs were recertified for 13 companies. While these certified reference materials are critically needed, their continuous production consumes considerable fiscal and human resources and limits our ability to address new standards needs. The gas NIST Traceable Reference Materials (NTRM) Program was created to address this concern. In FY01, we worked with 4 specialty gas vendors to certify 36 NTRM batches. The over 1000 individual gas cylinders in these certified batches will be used to produce approximately 100,000 NIST-traceable gas standards for end-users.

In the area of electrolytic conductivity, we continued our research into new packaging technology. The problem we face is that the packaging used in the past was not suitable for storage of conductivity solutions for more than one year. This has caused us problems in that each solution must be remade every year and uncertainties could not be lowered below 1%. During the past year we decided to package all conductivity solutions greater than 500 $\mu\text{S}/\text{cm}$ in 50 ml ampoules. We are still researching the problem for lower conductivity solutions. In sealed ampoules the transpiration problem, which has plagued us in the past, is solved and the solutions have multiyear self-lives. This should help us in the electrolytic conductivity program by reducing the SRM reissue rate so we can devote more time to low conductivity research, which is desperately needed to address needs in industry driven by regulations. Collaboration with the Netherlands (NMI) will help in this research. We have also benchmarked and compared our capabilities in the area of electrolytic conductivity measurements with the National Metrology Institutes for Denmark and

Hungary. The data from this benchmarking activity will appear in the journal of the BIPM, *Metrologia* in FY01. We are also participating in a CCQM pilot study on conductivity (CCQM-P22).

In the area of pH we continue to be very active in the international arena. In FY01 we participated in a pH Key Comparison (CCQM-K17), phthalate buffer (pH 4.0), and assisted the pilot laboratory (PTB) in preparing the solutions. We also completed a SIM pilot study on pH (SIM QM-P4) with 16 participating laboratories in South America, Mexico and the Caribbean. We also continue to be active in IUPAC commission V.5, completing revisions to the pH document that will define the traceability of pH to the Bates-Guggenheim convention, thus assuring continued traceability of pH to sound thermodynamic principals. This manuscript is now working its way through the IUPAC review process.

In collaboration with EPA and the remote sensing community, we have developed a quantitative database of infrared spectra, which is required for establishing remote IR-based technology as a reliable tool for real-time monitoring for airborne chemical contaminants along plant boundaries and within plant facilities. Because the spectra are being prepared using NIST primary gas standards, well-defined traceability to NIST can be established for any subsequent field measurements. These spectra will be required to be used in the new update of EPA method TO-16. At the present time we have released 40 compounds. We are working in collaboration with PNNL on an error affecting the higher wavenumbers, which deals with a fundamental problem with commercial instruments. This will result in an interesting paper that will detail needed changes in commercial instruments to resolve this systematic bias. In the near future we hope to increase user availability of this database on the Internet.

We continue to be very busy in the Ozone Program with completion of the upgrade of the EPA Standard Reference Photometer (SRP) network with new electronics. Many more countries have expressed interest in receiving an SRP to provide traceability in ozone measurements. New SRPs have been ordered by Portugal, Spain, BIPM, and Brazil. This presents a problem to NIST, in that we expend many resources to provide traceability to most of the world. It would be in NIST's interest to transfer this responsibility to another organization so we could shift those resources to areas of concern to U.S. industry. During the past year, we concluded talks, which will transfer the responsibility for world traceability for ozone to the BIPM in Paris, France. Over the next 5 years NIST will be working with BIPM to build two SRPs to act as the official ozone traveling standards, and a Primary Reference Photometer (PRP) to act as the stable international standard for ozone. Two PRPs will be constructed, one at NIST and one at BIPM. Work will be concluded in FY02 on construction of the two SRPs, and in design of the PRP. It is planned that construction of the PRP will begin in FY03. Plans for a CCQM key comparison on ozone are proceeding, and will be organized after BIPM has received their two SRPs.

We continue to promote U.S. industry through development of high priority standards, and through standards organizations such as ASTM. Over the past two years we have developed the capability to produce low concentration nitric oxide gas standards. These standards are needed by the automotive industry in new car development and to meet new regulations in California. These standards also are required by Industry to meet new regulations covering stack gas emissions. This research has resulted in two new nitric oxide gas SRMs in the coming years, one at 0.5 ppm (SRM 2737) and one at 1.0 ppm (SRM 2738).

In July 2001, a meeting was held at NIST with representatives from the American Industry/Government Emissions Research consortium (AIGER) to discuss research and standards needs to support emissions testing for the next generation of automobiles. AIGER is comprised of the California Air Resources Board, the US EPA, and the Automobile Industry (Daimler-Chrysler, Ford, General Motors). Critical new standards are needed for methane, propane, nitric oxide, and carbon dioxide that are significantly lower in concentration than SRMs currently available. These new standards will require research into new cylinder technology, and in new delivery systems in order to provide traceability. Without these new gas standards, delivery of lower emission automobiles will be impeded. We will be working closely with AIGER to develop the new standards, and to certify new NTRM lots that meet their strict requirements.

We also are continuing to assist the health industry with the task of safely implementing the inhaled nitric oxide therapy for newborns. Over the past year we have held another workshop to test clinical instruments, which measure nitric oxide and nitrogen dioxide. We have also assisted ASTM and industry draft a standard procedure to test these clinical instruments effectively before introduction into the hospitals and in continued operation.

Molecular Spectrometry and Microfluidic Methods Group

The MS&MM Group conducts research on the metrology of molecular spectrometry and develops standards for calibration, validation, and performance of instruments for measuring molecular spectra; conducts research on microfluidic devices, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in microchannels and the use of microchannel and other electrophoretic methods for forensic and toxicological applications and standards; has responsibility for the development and certification of optical transmittance and wavelength standards in the ultraviolet, visible, and near-infrared spectral regions; and works with users and manufacturers of analytical instruments to assess and measure the performance of analytical methods and to determine and address existing and future needs for analytical instrument standards ranging from device calibration and instrument performance through specifications for remote device control and data interchange.

In FY01, 68 solid absorbance filter SRMs were certified, and 176 optical filter sets were recertified. Continuing measurements were made on a number of other filter sets. In addition, 175 units of SRM 2034 (holmium oxide UV-Vis wavelength standard), 98 units of SRM 2065 (UV-Vis-NIR transmission wavelength standard), and 200 units of SRM 2241 (Relative Intensity Standard for Raman Spectroscopy with 785 nm Excitation) were certified. A new “just-in-time” filter certification process was instituted to solve the problem of filter certifications expiring prior to sale. We began to utilize the infrared laser marking system to etch identification numbers onto our optical filter glass and fused silica products—this year’s production run of SRM 2034 featured the SRM number and series identification etched into the fused silica cuvettes. We conducted a study to see if the laser etching would produce any measurable effect on the transmittance of our optical filters—it did not. The laser etcher was also utilized to label the anodized aluminum holders for SRM 2241.

Several new features were added to the high accuracy reference spectrometer (HAS-II) including capabilities to measure 50 mm (2 inch) square filters (useful for international intercomparisons), to determine filter transmittance at three height and multiple width positions on a filter (useful for filter homogeneity assessments), and to provide easy access to the data in the database for control chart generation. The effort to renovate the original high accuracy reference spectrometer (HAS-I)

continued with the development of a new integrating sphere, new sample transport, new visible lamp controller, new shutter and controller, and automation to drive the monochromator and monitor its position. The goal for HAS-I renovation is to reach the level of automation and control currently available for HAS-II so that HAS-I can be used for research on spectrometric measurements. Since HAS-II is used for production (certification and recertification) activities, it cannot be (and should not be) used for research.

Research was carried out to develop an extended set of visible filters (0.1%, 0.3%, and 70% transmittance) to enlarge the range of transmittances covered and fill in a gap in our coverage. This is being done to make the range of our visible filter offerings more compatible with those of other NMIs (e.g., NPL) and to meet partially requests by CORM, our NTRM vendors, and others for broadening the range of our standards. Modifications to the HAS-II software will be required before we can actually certify these darker filters. Our studies into causes of the problems plaguing the production of our metal-on-fused-silica filters yielded some solutions. The spectral interference (fringe) problem was solved by purposefully making the filter substrate and cover plate different thicknesses. The aging process (filter drift) was improved considerably by heating the filters after the contacting step. The remaining two difficulties, coating inhomogeneity and spectral slope (decreasing transmittance with decreasing wavelength) remain under study.

The NVLAP assessments and accreditations were completed for the three participating NTRM certifiers of optical filters, although only one of the three has passed the post-accreditation phase involving our testing of their production filters. Going into this year, the traceability aspect of this program was vague as to the actual methodology of assigning uncertainties and/or bias correction equations to the NTRM certifiers. We designed and implemented computer macros to massage comparative measurements made on SRMs, proficiency-test (PT) samples, and NTRM production "batch-test" filters, resulting in a tighter traceability link and lower uncertainties than anticipated. It appears that we can maintain traceability through the use of the batch samples sent to us for approval and the SRM recertifications. This will streamline the proficiency testing requirements and simplify the process for us and the certifiers. A fourth vendor has expressed a desire to join the program.

The NORAMET intercomparison of holmium oxide solutions for UV/Visible wavelength standards (see individual TAR) was expanded to participating SIM nations, and measurements were made in Trinidad and Tobago and Brazil this year. Although two participating countries remain for the SIM intercomparison, the NORAMET portion has been completed, and a draft of the report has been prepared. The analysis of the NORAMET data has very significant consequences for UV/visible wavelength standards. The first of these is the revision of our uncertainty estimates for SRM 2034. The second consequence is a decision to convert this standard from an artifact to an inherent (data) standard. The samples are now in Argentina, and we have scheduled the final shipments to Costa Rica when they return.

Progress was made on the creation of a NIR wavelength "transflectance" standard. Test samples from the production block of glass were cut, polished, and evaluated in transmission mode. The temperature coefficients of the material were determined. The next step is to mate the glass with the polyfluorocarbon diffuse reflector to create the reflectance/transflectance device that will be certified as the SRM.

The certification of first Ramen relative intensity correction standard for excitation at 785 nm was completed this year (see separated TAR on SRM 2241). Suitability studies were completed on the

second material, a uranium glass for excitation at 488 nm to 532 nm. This paves the way for the certification of SRM 2242 in the upcoming year. Studies were also conducted on an europium-bearing glass for 1064 nm excitation (SRM 2243).

We continued our search for candidate materials for luminescence standards and examined several transition-metal-, rare-earth-, and actinide-doped glasses. Uranium- and manganese-bearing glasses look promising for fluorescence spectral emissivity standards. We are also studying glasses containing mixtures of rare-earth s as possible fluorescence wavelength standards. Unlike absorbance, mixed rare-earth glasses do not give fluorescence spectra that are simply the sum of the spectra of the separate element-doped glasses. With mixed elements, emission bands can decrease in intensity, grow in intensity, shift in wavelength, and/or disappear altogether. Elemental concentration, the type of glass matrix, and the glass making conditions all have effects on the final fluorescence spectra observed.

We continued to qualify the SPEX-JY fluorometer. We recently had a small integrating sphere/photodiode assembly calibrated in PL. This will allow us to quantify the light from the excitation monochromator. We fabricated several sample chamber devices to hold samples, to reflect light into the emission monochromator, to hold tritiated phosphor devices, etc. These will be used to further qualify the instrument and determine its stability, etc. A standard uniform light source has been constructed to calibrate the emission detection system. After further testing, it will be sent to PL for calibration.

Progress was made on linking the fluorometer to the dual diode array detector system that will allow us to obtain excitation-emission matrix spectra rapidly. Using the fluorometer's built-in optics, the detected signals proved weaker than desired; accordingly, an alternate optical arrangement is being investigated. Devising the software to control both instruments simultaneously remains the biggest hurdle.

We continued to provide statistics and data representation studies for FBI, OLES, and other agencies investigating the use of DNA methods of forensic analysis including studies on various aspects of STR DNA typing, particularly related to how errors in the measurement of DNA quantity propagate into the detection of the STR profile and the recovery of DNA from aged stains; on the quantitative characterization of STR allelic signals and assignment of match thresholds; and on aspects of gunpowder residue analysis. We continued to provide computational, organizational, and philosophical support for the Micronutrients Measurement Quality Assurance Program including data analysis and feedback for reports on two fat-soluble and one vitamin C interlaboratory studies.

Our project, initiated through the Office of Law Enforcement Standards at NIST, to advance and support quantitative measurements for forensic analyses received a boost this year from the symposium we organized and chaired at PittCon. The program was both well received and well attended. A report about it was featured in a subsequent PittCon promotional brochure. Progress was made on the first gunpowder reference material (RM 8107). A single ball rifle powder has been selected as the material, and the procedure for extracting and quantifying the organic additives from this material was developed. Our OLES-sponsored forensic analysis fellowship program continued with support for two graduate students this year. The MECE procedure that we developed for gunpowder additives was recently used in criminal case work by the Washington State Patrol and has also been adopted by the Army Research Lab at the Redstone Arsenal for propellant additive determination.

This year was a banner year for our microfluidics projects resulting in eleven publications, five patent applications, and six invited talks (see separate TAR). Microfluidics funding, which had been largely from the Microscale Analytical Laboratory Competence, a small ATP grant, and STRS, received additional ATP sponsorship this year and will receive an additional boost from a new Single Molecule Manipulation and Measurement competence award. The joint program with Div. 836 continues to attract NRC postdocs, adding one more this year to bring the MS&MM total to three. One of them, Emanuel Waddell, won first prize in the student/postdoc poster contest at LabAutomation 2001.

We continue to study the parameters that affect flow in plastic microchannel devices. This year major progress was made in exploring applications of UV-laser ablation for fabricating microdevices, for studying parameters important to the post-machining properties of microdevices, and for carrying out both physical and chemical modifications to the surfaces of microdevices. New techniques were discovered for controlling surface charge and the spatial distribution of surface charge in channels made either by imprinting, laser ablation, or hybrid fabrication techniques using both methods. Extensive progress has been made in evaluating flow in microchannels using particle velocimetry and caged fluorescent dye imaging. Thanks to the availability of ITL-procured simulation software, we have begun to model flow in microchannels. These simulations have already been beneficial in new fluid mixer and flow steering designs. The microfluidics projects are rich in collaborations—across NIST and off-campus in both academe and industry. In one such collaboration with Cornell University, a liposome-based microfluidic assay was developed for detection of RNA/DNA in *Cryptosporidium parvum*, a difficult to kill environmental pathogen.

Working with Guest Researchers for the Fachhochschule in Wiesbaden Germany, we developed SpectroML a markup language for molecular spectrometry data that can be used as a “web-aware” mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange and archiving (see separate TAR). SpectroML was developed using XML (extensible mark up language), and its vocabulary was gleaned from the terminology, data dictionaries, and concepts embodied in existing standards, instrument software, and data interchange formats. Currently, we have created a SpectroML vocabulary, document type definition, schema, stylesheets, and some demonstration applications for UV-visible spectroscopy data; however, the structure and flexible data model embodied in SpectroML should make it easily adaptable to other spectroscopy techniques. We are currently developing applications to utilize SpectroML in house for data interchange among our spectrometers and also those of our NTRM vendors.

Nuclear Analytical Methods

Research activities in this group are focused on the science that supports the identification and quantitation of chemical species by nuclear analytical techniques. Current laboratory research activities involve the full suite of nuclear analytical techniques, including instrumental and radiochemical neutron activation analysis (INAA and RNAA), prompt gamma activation analysis (PGAA), and neutron depth profiling (NDP). In addition, we are developing analytical applications of neutron focusing technology. The measurement capabilities that reside within this group provide an excellent complement to those in the Spectrochemical Methods Group in that they depend upon characteristics of the nucleus of the element rather than the electron shells, and therefore are insensitive to the chemical state of the analyte. In addition, the nuclear analytical methods are generally nondestructive and do not require sample dissolution, thus providing an independent assay.

INAA and RNAA are powerful reference techniques that have been used at NIST for many years. New developments continue to provide improvements in detection sensitivities, elemental specificity, precision, and overall accuracy that allow nuclear methods to address new measurement needs. We have recently developed new INAA procedures and applied them to homogeneity studies of SRMs at small sample sizes. Many analytical techniques used in industry and academia rely on the analysis of very small samples (i.e., 1 mg), typically in the solid (undissolved) form. Unfortunately most SRMs are certified with minimum sample sizes of 100-500 mg, and are therefore unsuitable for use as control materials for these techniques unless additional information is made available. Taking advantage of the sensitivity and nondestructive properties of INAA, the use of this technique for homogeneity studies of small samples has been evaluated and implemented for the determination of sampling characteristics for a number of environmental SRMs. The minimal analytical uncertainty associated with the INAA measurements allows extraction of the variability due to material inhomogeneity from the observed total variability within a given set of measurements. We have investigated three sediment materials that have shown the potential for a high degree of homogeneity at very small sample sizes for possible use as a future SRM aimed at solid-sampling analytical techniques. From the candidate materials a portion of sediment from the Baltimore Harbor, currently under certification for “normal” sample sizes (SRM 2102) was selected for further processing as an SRM for microanalytical techniques. Evaluation of the Ingamells model describing homogeneity has been initiated with the Statistical Engineering Division.

Instrumental neutron activation analysis has been used as a primary method for certification of the arsenic implanted dose in SRM 2134, Arsenic Implant in Silicon. This material has been a high-priority need of the semiconductor industry for a number of years. It was produced and characterized in collaboration with the Surface and Microanalysis Science Division and is intended for use as a calibrant for secondary ion mass spectrometry. The INAA results used for certification include the first complete, quantitative evaluation of all sources of uncertainty in an INAA measurement. The expanded relative uncertainty for the mean value of this SRM was 0.38 % and approximates the 95 % level of confidence.

In the course of our high-accuracy measurements of arsenic, we found that the most recently published and compiled half-life of ^{76}As did not describe our data as well as the earlier accepted value. We have redetermined this parameter using four Ge detector systems, and fitted an exponential to the decay data by two different nonlinear least-squares methods. We measured $t_{1/2} = 1.0938$ d with an expanded uncertainty $U = 0.0009$ d. This result is 1.5% higher than the recent value, and in agreement with the older, less precise, consensus value.

As part of our attempts to demonstrate that instrumental neutron activation analysis meets the CCQM definition of a primary ratio method of analysis, we are currently determining the concentration of Cr in a ferrous metal, SRM 1152a. We will provide a complete uncertainty evaluation as in the case of arsenic in silicon. Analysis of a metal matrix challenges the INAA measurement process in a totally different manner than does determination of arsenic in silicon. The high density of the metal increases the magnitude of both neutron and gamma ray self-shielding. The metal matrix also produces an elevated level of baseline radiation. An additional reason we have chosen to determine Cr in SRM 1152a is the small uncertainty in its certified value. Initial measurements were conducted using crystalline chromium metal as a comparator. The measurements were validated with weighed aliquots of SRM 3112a dried on filter paper pellets. The experimental results do not show deviations beyond the uncertainties of the SRMs (≤ 0.2 % relative), and the initial assessment of the uncertainty budget indicates that an expanded uncertainty of ≤ 0.3 % should be achievable.

Radiochemical neutron activation analysis has proven to be a powerful tool for measuring trace phosphorus in a variety of materials. An RNAA procedure has been developed to determine and value assign phosphorus in various SRMs. Phosphorus is quantified by beta counting of ^{32}P after radiochemical separation. Originally developed for the analysis of metals, the method was used to value assign phosphorus in SRMs 861 (Aircraft Superalloy) and 2175 (Refractory Alloy), both containing phosphorus at low mg/kg levels. Modifications to the procedure have allowed us to value assign phosphorus in two new SRMs: 1575a (Pine Needles) and 2702 (Inorganics in Marine Sediment). Plans are currently underway to develop an ion-implanted phosphorus in silicon SRM for the semiconductor industry, which will be value assigned by RNAA. The RNAA procedure has been modified for use in determining phosphorus in implanted silicon, critically evaluated, and applied to subsamples of an ion-implanted phosphorus in silicon wafer previously used for an interlaboratory comparison. Our RNAA results indicate that phosphorus can be determined at approximately 8.5×10^{14} atoms \cdot cm $^{-2}$ with an expanded uncertainty of 3 %.

Nearly thirty years ago a simple and rapid radiochemical procedure was developed at NIST, using oxygen combustion to separate mercury quantitatively from interfering radioactivities. Mercury was subsequently determined by counting the 64-hour ^{197}Hg with a low-energy Ge gamma detector. This method was used extensively for the certification of mercury in biological materials and other reference materials. However, the published combustion method suffers from problems due to high levels of activity from ^{82}Br , which elevates the baseline level under the peaks of interest. This radiochemical procedure is not optimum for determination of mercury via 47-day ^{203}Hg , because selenium follows mercury efficiently in the combustion (indeed, an almost identical procedure was developed for selenium determination). The sole ^{203}Hg gamma is interfered with by 120-day ^{75}Se , so correction for this interference is imprecise in high-selenium matrices. In the present work selenium is removed by precipitating mercury as periodate. The chemical yield of each sample is measured gravimetrically. The procedure has been applied to the certification of mercury in SRMs 1632c Coal, 2702 Sediment, and 1575a Pine Needles. Expanded uncertainties of 2 to 3 % can be achieved via this method.

Pioneering research is conducted by the Nuclear Methods Group on the use of cold neutron beams as analytical probes for both PGAA and NDP. PGAA measures the total amount of an analyte present throughout a sample by the analysis of the prompt gamma rays emitted immediately following neutron capture. NDP, on the other hand, determines the concentrations of several important elements (isotopes) as a function of depth within the first few micrometers of the surface by energy analysis of the prompt charged particles emitted during neutron bombardment. Both of these techniques continue to make important contributions in the characterization of advanced materials, particularly with the enhanced sensitivities now available using cold neutrons from the NIST Reactor.

The cold neutron PGAA and NDP spectrometers, which are available to outside researchers as part of the NIST Center for Neutron Research “national users’ facility”, continue to provide high-quality measurements for outside scientists. Non-destructive, matrix-independent measurements of hydrogen by the cold neutron PGAA technique have become particularly valuable in view of the difficulties of accurate determination of this element by other techniques. Many of our current PGAA collaborations involve determining hydrogen in a wide variety of materials for different applications. These include: metal embrittlement studies, studies of materials for new types of lithium batteries and fuel cells, determination of hydrogen in porous thin films on silicon, and screening materials for H prior to neutron scattering measurements. Other measurements made at

the PGAA facility this year include H, S, Ca, and K in Nafions, derivatives of Teflon which have potential use as membranes in electrochemical separations and in fuel cells; Dy in polymers with potential use in optical communications, Bi, Nb, and Zn in dielectric materials with potential in wireless communications, and La, Sr, Cu, and Zn in antiferromagnetic materials which are of interest to the superconductor industry. In a continuing project, cold neutron PGAA is being used in conjunction with crystallography to study the stoichiometry and arrangement of the atoms in zeolites, acid based catalysts used in production of gasoline. PGAA is being used to measure H, Al, and Si in these materials, and also to quantitatively measure deuterium/hydrogen exchange.

A method has been developed and apparatus built to produce titanium (and other metal) SRMs of known hydrogen concentration on the few-kilogram scale. The method is based on the controlled reaction of hydrogen with titanium in a closed system. After preparation, the hydrogen concentration is verified by cold-neutron prompt-gamma activation analysis and gravimetry. The first SRM, 2453 (Hydrogen in Titanium Alloy) has been prepared near the critical level of approximately 100 mg/kg. Cold neutron PGAA has been used to measure a hydrogen mass fraction of 114 ± 14 mg/kg, in agreement with the volumetric measurement. Two additional hydrogen in titanium SRMs will be prepared at bracketing levels. This apparatus has also been used to prepare standards for neutron-tomographic nondestructive analysis of turbine blades at McClellan Air Force Base.

We are currently developing and critically evaluating a second nondestructive technique for the quantitative determination of hydrogen based upon neutron incoherent scattering (NIS). This will serve as a complement to our PGAA measurements of this element. NIS has a limited overlap of error sources with PGAA, and agreement of results between the two techniques provides a high degree of confidence. In addition, by measuring the scattered neutrons using a position-sensitive detector, NIS may be used to determine the location of hydrogen within various metallic systems. Using a slit or aperture in the scattering geometry, we have demonstrated the imaging of hydrogen in layers of polypropylene sandwiched between sets of titanium plates, and in titanium standards containing known amounts of hydrogen. We have shown the ability to image hydrogen in titanium at the 100 $\mu\text{g/g}$ level as a function of location. Analysis of the images shows that the scattering from the hydrogen increases linearly with its mass fraction within the titanium.

In collaboration with the Reactor Radiation Division (856) and the US Food and Drug Administration, a new thermal neutron prompt gamma ray activation analysis instrument was designed and fabricated. This instrument became operational in FY01. A sapphire filter was placed in the neutron beam shutter assembly and all external components of the original system were replaced. The new beam tube is made of two sections constructed from aluminum lined with a lithiated polymer (lithoflex). Both sections are sealed at each end, and kept under vacuum. The sample chamber was also constructed of aluminum and lined with lithoflex. The chamber may be evacuated when necessary. The new Ge detector has a resolution of 2.0 keV (for the 1332.5 keV line from ^{60}Co) and 40% efficiency (relative to NaI). Experiments were performed to determine mass fractions of Cd in SRM 2702 Sediment, and B, K and Cl in SRM 1575a Pine Needles. Additional experiments were performed to determine the background count-rates during various operational conditions, to measure element sensitivities in this configuration, and to determine the optimum amount of neutron and gamma ray shielding that will be incorporated into the final design for the detection system. A variable-position detector support platform will be designed. Additional work will involve optimization the detection system and shielding configuration, and complete characterization of the new system.

A novel approach is under investigation to alleviate some persistent problems in PGAA. Detection sensitivities of this technique are often restricted by the following factors: poor signal-to-noise ratios, interferences from background signals, and, in some cases, overlapping energy lines from different origins, namely ultra-short lived decay lines interfering with prompt decay. Discrimination between prompt and delayed emissions from a sample source, as well as against background events, can be achieved by examining the timing between the gamma-ray acquisition with the actual capture events using a pulsed beam of cold neutrons. Coincidence gating is used to select the prompt gamma-ray emissions. Signals from background capture gamma rays are suppressed because of times at which gamma rays are measured can differentiate between the sources of background radiation, providing a reduction in direct gamma-ray interferences. Anti-coincidence gating allows measurement of only decay radiation that originates from short-lived activated states of the nuclides after capture. Spectra of decaying nuclides are free of interfering prompt activities, and also have lower continuum background from Compton scattering of high-energy prompt gamma rays in the detector. The measurements provide the opportunity to use ultra-short half-life nuclides for analytical purposes; no sample decay occurs during transfer times, and repetitive activation and counting cycles are achieved with the use of pulsed neutron beams.

The neutron depth profiling facility continues to provide unique measurement capabilities directly to US industries. Current experiments of interest at the NDP instrument include the measurement of lithium concentration and distribution in thin films being studied for battery applications, studies of boron mobility in tungsten with the Army Research Laboratory, shallow-doped boron content in silicon in conjunction with Advanced Micro Devices, the study of lithium distribution in lithium niobate, and the measurement of nitrogen in layers such as TiN and GaN. As a recent example, we have used NDP to measure nitrogen distributions in GaN/GaAs bilayers with Corning. This material is a base material for construction of devices such as blue light emitting lasers. The N concentration, which we can determine quantitatively, is a crucial parameter for establishing the device characteristics.

As part of a continuing collaboration between NIST and Tufts University, we have employed NDP to measure elemental concentrations versus depth for different lithium ion battery materials. One goal is to rationally design the thermo-mechanical properties of amorphous lithium phosphorus oxynitride (lipon, a solid ionic conductor), particularly the thermal stress (thermal expansion coefficient). A part of this study is to relate the resulting thermal stress to starting composition and temperature of evaporant, and of the composition and pressure of the background gas during deposition. We are also studying LiCoO₂ films (a material that can be used as an electrode in batteries) to determine if the ion beam assisted deposition process being used has the capability of controlling not only the degree of crystallinity and orientation of crystallites, but also the Li/Co ratio.

We have just concluded an assessment of long term stability of primary boron and lithium thin film standards for NDP. Results show that the lithium standards have not changed over a ten year period, while the boron standards have lost from 0.1 to 0.5% of their original value. This will guide us in the design parameters of the next set of boron standards.

Efforts continue within the group to develop the techniques and methodology of focusing cold neutron beams for analytical applications of PGAA and NDP. A monolithic lens consisting of a fused tapered bundle of polycapillaries that provides a smaller focus of about 0.15 mm (FWHM at the focal spot) has been installed in the NDP chamber. The chamber has also been modified to accept a separate lid on which the lens can be mounted for remote-controlled alignment. A new

alignment system containing a CID neutron camera with vacuum capability has also been installed. It provides a spatial resolution of 12 μm (pixel size) and an 8 bit dynamic range for the neutron intensity, and provides the possibility for charged particle imaging. Together with the new vacuum-compatible motion stages, the alignment system permits measurements without breaking the vacuum. Furthermore, the lens can be retracted out of the beam for measurements on the same sample with normal NDP geometry. The new lens and focusing system now gives us the capability of measuring samples as small as ≈ 0.1 mm in diameter or, together with our current scanning system, of measuring concentrations in three dimensions with much improved spatial resolution. Previously, our three dimensional profiles were performed with beam spot sizes of 1 cm.

Neutron focusing lenses have been shown to enhance the measurement capabilities of prompt gamma activation analysis (PGAA) for small samples (≈ 100 μm in size) using a reactor-based cold neutron beam. Previously, a cold neutron beam emerging from a ^{58}Ni -coated guide, cross section = 50 mm x 45 mm, has been compressed to a spot size of about 0.54 mm (FWHM). Recently, we have made preliminary prompt-gamma measurements using a monolithic capillary lens. The lens accepts a 10 mm (hexagon flat-to-flat) size beam and focuses it to a spot size of < 100 μm x 100 μm (FWHM), with a gain in neutron beam current density of > 40 . The smaller focal spot size enables better spatial resolution, but also makes sample alignment more challenging. We have added a neutron imaging technique to the sample positioning procedure that takes advantage of the converging and subsequent diverging nature of the focused beam. The measurement sensitivity for a 2.6 μg Gd sample has improved by a factor of 34. In addition to rastering samples in the lateral plane, we have also explored the possibility of profiling the inhomogeneity of the sample in the direction along the beam axis.

A neutron thermometer technique has been employed to characterize neutron beam spectra at various instruments. The idea is to measure the prompt gamma signals from a “thin” (negligible absorption) and a “thick” (total absorption) sample, and to deduce the effective absorption cross section with the average neutron wavelength. This information is useful for characterizing “white beam” measurements such as those used for NDP and PGAA, as well as when additional components are introduced in the beam (such a filter or a neutron lens), which alters the neutron spectrum.

A technique for performing pseudo-Laue neutron crystallography of small single crystals with large unit cells, such as proteins, has been investigated using polycapillary optics. Instead of using a broad wavelength band, the technique uses a broad angular bandwidth in order to increase the neutron current density on the crystal. The convergent beam results in elongated diffraction spots that require modified software for analysis. We have taken measurements to characterize the transmission of a monolithic focusing lens with a focal length of 100 mm that was later used for preliminary tests of the proposed neutron technique on a pulsed source. The alignment of the optic in the neutron beam is most critical.

Reference Methods and Standards for Food and Nutrition

CSTL Program: Healthcare Measurements

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Abstract: The Analytical Chemistry Division (ACD) is working in several areas within the broad category of “food and nutrition” to facilitate compliance with nutritional and dietary supplement labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices. The areas in which ACD is working include development of reference materials and analytical methods for nutrition labeling applications, dietary supplements including botanical materials, and genetically modified organisms. A number of food-matrix reference materials with values assigned for nutrient concentrations are now available across a wide spectrum of food compositions. NIST has begun to focus on more specific interests in the food area such as dietary supplements (particularly botanicals) and genetically modified foods to impact manufacturers and consumers of these products.

Purpose: To develop reference materials and analytical methods to underpin measurements made for compliance with nutritional and dietary supplement labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices

Major Accomplishments:

Nutrients

The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed. AOAC (Association of Official Analytical Chemists) International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC’s belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods. NIST has been working with other government agencies and the food industry over the past several years to provide an increased array of SRMs with values assigned for proximates (procedurally defined values for fat, protein, carbohydrate, etc.), fatty acids, cholesterol, vitamins, elements of nutritional interest, etc. Concentration values in the food-matrix SRMs are assigned based on a combination of data from NIST, from interlaboratory comparison exercises involving approximately 20 member laboratories of the National Food Processors Association’s (NFPA’s) Food Industry Analytical Chemists Subcommittee (FIACS), and from additional collaborating laboratories.

Value-assignment of two food-matrix SRMs was completed this year. Their positions, along with those of the other food-matrix reference materials available from NIST, are shown below. SRM 1946 Lake Superior Fish Tissue is a cryogenically homogenized fish fillet collected from adult lake trout (*Salvelinus namaycush*). This material has values assigned for nutrients including proximates (fat, protein, etc.) and individual fatty acids, including several omega-3 fatty acids. SRM 2384

Baking Chocolate has values assigned for proximates, individual fatty acids, total dietary fiber, several vitamins, and elements of nutritional interest.

Contaminants

NIST has SRMs available for cod liver oil (>90% lipid), mussel tissue (<1% lipid), and whale blubber (~70 % lipid) but has received a number of requests for a fish tissue (~10% lipid), particularly from programs that routinely monitor different species of fish, both for environmental concerns and nutritional value, as described above. Thus, SRM 1946 Lake Superior Fish Tissue was produced. Levels of organic contaminants in this material are indicative of urban contamination levels and will be beneficial to both the environmental and food/nutrition measurement communities. Certified values will be available for 30 polychlorinated biphenyl (PCB) congeners, including 3 non-ortho congeners, 13 chlorinated pesticides, methyl mercury, and total mercury. This is the first SRM available with certified concentrations for non-ortho PCB congeners that are considered the most toxic, similar in structure to polychlorinated dibenzo-*p*-dioxins.

Nutraceuticals

The U.S. 1998 market for nutraceuticals (i.e., herbal supplements and functional foods) was estimated at nearly \$26 billion; botanicals accounted for about \$3.5 billion of this market, and a continued growth rate of 10% to 20% was expected. However, in 1998 sales of botanical products began to level off, and by the end of 1999, overall sales had dropped 3% compared to 1998. Part of this decrease in sales may be attributed to a loss in consumer confidence in botanical products as a result of negative publicity. Reports of inaccurate labeling, adulteration, contamination (pesticides, heavy metals, or toxic botanicals), and drug interactions have all contributed to the decline in sales. Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds, as well as for contaminants such as pesticides, mycotoxins, and heavy metals, are necessary for verification of manufacturers' label claims and quality control during the manufacturing process.

NIST representatives have been working with the AOAC's Dietary Supplement Task Group to identify nutraceuticals for which validated analytical methods are needed. It is useful to have a reference material when validating an analytical method. FDA representatives also providing funding to this task group, therefore the supplements identified as those for which methods are needed are likely to be the same as those for which reference materials are needed.

The Analytical Chemistry Division has developed a collaborative program with the Food and Drug Administration's (FDA's) Center for Food Safety and the National Institutes of Health's (NIH's) Office of Dietary Supplements to produce a number of authentic, well-characterized reference materials of botanical raw materials to support the needs of the dietary supplement community. ACD, FDA, and NIH plan to collaborate to prepare six to eight selected botanical-matrix SRMs from authentic raw materials during the next several years. NIH has provided funding and FDA has committed to provide funding starting in FY02. FDA, with input from other agencies and organizations, will consult with NIST to identify the materials of high priority.

Genetically modified organisms

Crops have been genetically modified to resist pests and disease, to tolerate chemical herbicides, to resist poor soil and weather conditions, or to possess improved nutritional content, food processing characteristics, ripening, texture, or flavor. Genetically modified seeds accounted for \$1.5 billion in sales in 1998, and genetically modified seeds were used to plant more than 70 million acres around the world (excluding China) – 50 million of these in the U.S. Agronomists have been breeding plants for centuries, and the FDA recognizes no difference between this and current practices. No labeling of genetically modified products is required in the U.S. unless the resultant product's nutritional content or allergenicity differs from the “normal” product; FDA is currently developing *voluntary* guidelines for labeling. However, labeling is currently mandated in the European Union, Japan, Australia, and New Zealand, and labeling regulations in South America are pending. The GMO issue is becoming a significant barrier to trade. Market disruptions in the EU have impacted U.S. corporations attempting to do business there.

Currently, the USDA's Grain Inspection, Packers, and Stockyards Administration (GIPSA) is establishing a reference laboratory that will evaluate and accredit PCR laboratories that test for GMOs in grains and oilseeds. These laboratories provide qualitative analyses. GIPSA is also evaluating the test kits that are available to determine the presence or absence of GMOs at certain levels. These test kits include ELISA (enzyme-linked immunosorbent assay)-based kits, which may be quantitative, as well as lateral flow strips, which provide a yes/no answer, much as a home-pregnancy test kit does. These test kits provide rapid results, but cannot adequately sample entire truckloads of grain. NIR spectrometers can be modified to examine as many as 1000 kernels or beans per test, and would be more appropriate for truckside testing.

GIPSA has been working with the Institute for Reference Materials and Measurements (IRMM) for production of GMO reference materials. Reference materials for many of the GMOs in GIPSA's proficiency testing program are now available from IRMM, and IRMM is currently constructing clean facilities for production of additional materials. Representatives from IRMM appear to be willing to collaborate with NIST in the production and value-assignment of additional materials to ensure that results worldwide are traceable to just one set of materials and to avoid further complicating an already complicated issue. Production of a series of GMO reference materials in conjunction with IRMM would allow GMO measurements in the US and Europe to be traceable to a single standard recognized by the governments of all parties, and could facilitate international trade by demonstrating the absence of GMO grains at the regulated threshold levels.

ACD has organized a workshop to be held at the Interamerican Metrology System's General Assembly Meeting to discuss the legislation that is currently in place within the Americas and in other geographic regions, the analytical methods that are currently available to address the legislation, and whether there is a gap between the two that needs to be addressed.

Impact: A number of food-matrix reference materials with values assigned for nutrient concentrations are now available across a wide spectrum of food compositions. These materials will ideally improve the accuracy of nutrition information provided on product labels. NIST has begun to focus on more specific interests in the food area such as dietary supplements (particularly botanicals) and genetically modified foods to impact both manufacturers and consumers of these products.

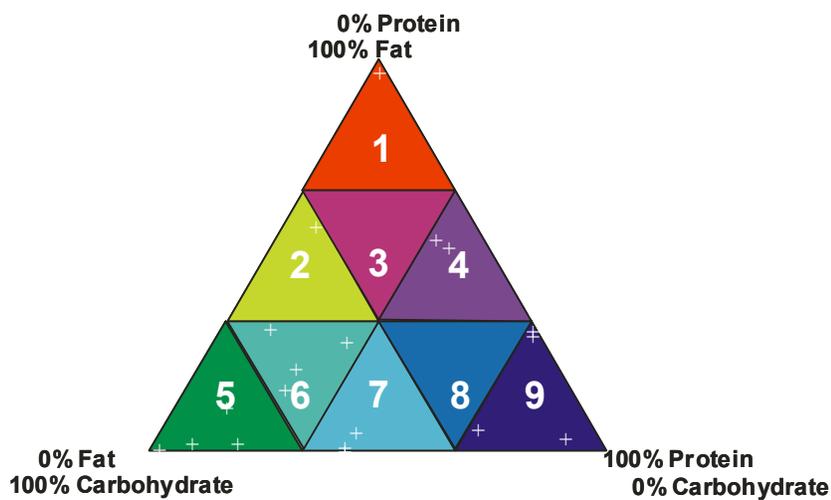
Future Plans: Two food-matrix SRMs are currently in production: SRM 2385 Spinach and 2387 Peanut Butter. Values are expected for nutrients, carotenoids, and pesticides in the spinach. Values are expected for nutrients, allergens, aflatoxins, and amino acids in the peanut butter. Following the release of these two materials, nutrition information will be available for at least one reference material in each sector of the AOAC triangle. This particular aspect of the food/nutrition component of NIST's programs can be expected to decrease. Focus will then shift to more specific analytes such as individual amino acids, allergens, and mycotoxins in foods, as well as botanical materials.

Many of the materials in the suite of food-matrix SRMs were developed as a result of needs assessments workshops in which participants from the food industry and regulatory agencies were asked to identify priority reference materials. These workshops, held in 1997 and 1999, led to the development of SRM 2384 Baking Chocolate, SRM 2385 Spinach, and SRM 2387 Peanut Butter; the forth-coming addition of amino acid information to SRM 1546 Meat Homogenate; and the inclusion of allergen and aflatoxin information with SRM 2387. Many members of the food industry are involved in the production of "functional foods," a category of nutraceuticals, and NIST's involvement with dietary supplements has also been identified as a high priority, and work in this area is ramping up with the support of FDA and NIH.

Food Triangle

<u>Sector</u>	<u>SRM Coverage of Food Triangle</u>
1	SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil
2	SRM 2384 Baking Chocolate
3	SRM 2387 Peanut Butter
4	SRM 1546 Meat Homogenate (<i>recently completed</i>)
5	SRM 2383 Baby Food Composite (<i>recently completed</i>)
6	SRM 1846 Infant Formula (<i>recently completed</i>) SRM 1548a Total Diet (<i>recently completed</i>) SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite
7	SRM 1566b Oyster Tissue (<i>recently completed</i>) SRM 1570a Trace Elements in Spinach Leaves SRM 2385 Spinach
Junction of 8, 9	SRM 1974a = Mussel Tissue
Junction of 4,8,9	SRM 1946 Lake Superior Fish Tissue SRM 1947 Lake Michigan Fish Tissue

Fig. 1. Populated AOAC food triangle with food matrices chosen by stakeholder



Methods and Standards for Commodities Characterization

CSTL Program: Chemical Characterization of Materials

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Abstract: The industrial engine of the United States runs on the production and commerce of many very basic commodities, including fossil fuels, steel (and other metal alloys), and cement. In fact, these three commodities represent the top three categories of Standard Reference Materials certified for chemical composition by NIST. The value and function of these commodities are strongly related to their chemical composition and chemical analysis is the basis of their trade. Since they are produced and traded in great volume, these chemical analyses tend to be done by high throughput, inexpensive instrumentation that rely on high accuracy standards for their calibration and validation. The importance of standards and standard methods for these materials is reflected in the prominence of the American Society for Testing and Materials (ASTM) committees for these industrial sectors. NIST works closely with ASTM, as well as other industrial groups to help define the highest priority measurement and standard needs for these basic commodity sectors. For instance, ASTM Committees C01 Cements, D02 Petroleum Products and Lubricants, D05 Coal and Coke, and E01 Analytical Chemistry for Metals, Ores, and Related Products, and others work with NIST for new standards development.

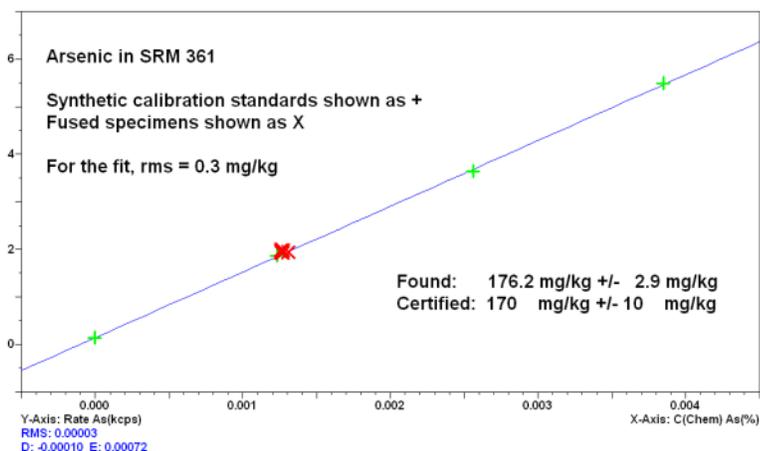
CSTL has developed new methods and standards to respond to these industries' needs. These include new hydrogen, sulfur, phosphorus, and nitrogen in metals standards. A new matrix independent method X-ray Fluorescence method has been developed that has been used for metals and cements, as well as a definitive method for Hg in Coals. Titanium dioxide has been recertified using an absolute coulometric method.

Purpose: To develop and apply methods and develop standards that underpin accurate and traceable commodity analysis in the United States.

Major Accomplishments:

Metals:

1) Matrix-independent X-ray fluorescence (XRF): This method provides direct traceability to primary reference materials in combination with the speed and repeatability of XRF. The complete matrix-independent concept was implemented for low alloy steel as a demonstration case. There are several parts to the method: 1) sample preparation by acid reaction and borate fusion; 2) demonstration of performance for repeatability, sensitivity and detection limits; and 3) calibration using primary reference materials. Chip form, low alloy steel SRMs 361 – 365 were fused after reaction with HNO₃ and H₂O₂. Relative standard deviations of replicate fusions



were less than 1 %. The same alloys are available in disk form as SRMs 1261 – 1265. Calibrations for 20 elements were created using a straight-line model for both fused and disk forms. Borate fusion does not sacrifice sensitivity equal to the dilution factor because bulk Fe absorbs X-rays about three times more strongly than Li borate beads. Fusion diluted the chip form SRMs by a factor of 13.4, but sensitivity loss was only a factor of 4, at worst, and detection limits (original sample basis) were higher by a factor of only 3, at worst. Calibration by synthetic, bracket standards (shown) was used to analyze SRM 361 and resulted in excellent performance even for trace arsenic (plot), normally considered a volatile element.

This method provides direct traceability to primary reference materials for a segment of the metals industry that has few certified reference materials. In general, XRF is no longer limited to disk form specimens, which is especially important for alloys that do not form homogeneous solids. In fact, the superior speed and repeatability of XRF can be used to greater advantage for metals, alloys, ores and related materials with fewer certified reference materials.

2) Certification of SRM 2453 Hydrogen in Titanium. SRM 2453, the first of a projected series of three Standard Reference Materials of trace hydrogen in titanium alloy has been completed. The certified hydrogen concentration will be 114 mg/kg, with an expanded uncertainty of 5 mg/kg. This Standard Reference Material (SRM) is intended for use in the evaluation of methods and the calibration of equipment used in the determination of hydrogen in titanium alloy. Plans for the FY02-03 call for the production of two additional materials at nominal concentrations of 50 and 200 mg/kg.

This SRM was prepared and analyzed at NIST. Sheets of Ti90/Al6/V4 alloy were cleaned, weighed, and degassed at temperatures up to 800°C in a high-vacuum furnace system. The degassed material was sampled, reweighed, and returned to vacuum. A measured quantity of hydrogen was added to the system from a calibrated volume, and hydriding accomplished at elevated temperature. The samples were analyzed by cold-neutron prompt-gamma activation analysis (PGAA) and neutron incoherent scattering (NIS). The batch was chipped to a particle size of approximately 15 mg to improve homogeneity. The chipped material was cleaned by extraction with methanol, dried, blended, and bottled. Cold-neutron prompt-gamma activation analysis (PGAA) was used to determine the hydrogen concentration. The certified value is the sum of the residual concentration in the blank material (measured by PGAA and NIS) and the quantity of hydrogen added as determined by volumetric measurements.

3) Low-Level Phosphorus in Metals: A radiochemical neutron activation analysis (RNAA) procedure has been developed to measure low-level phosphorus in metals. Samples are first irradiated at a neutron flux of $3 \times 10^{13} \text{ cm}^{-2}\text{s}^{-1}$. During sample irradiation, ^{31}P undergoes neutron capture to form ^{32}P , a pure beta emitter with a half life of 14.28 days. After irradiation, samples are mixed with inactive phosphorus carrier, then dissolved in a mixture of acids. Phosphorus is separated from the matrix and purified of other radionuclides by cation exchange, followed by precipitation of phosphorus as ammonium phosphomolybdate, then as magnesium ammonium phosphate. The yield (fraction of recovered carrier) is determined gravimetrically as $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$. ^{32}P is measured using a beta proportional counter. Comparison of sample count rates and carrier yields with those of standards processed in a similar manner allows calculation of P mass fractions. The detection limit for a 0.1 g sample irradiated for 30 minutes is 5 µg/kg. The procedure was originally developed for and critically evaluated through analysis of low alloy steels with P mass fractions ranging from 100 to 400 mg/kg. A slightly modified version of the original procedure was used to value assign phosphorus in two low phosphorus metal alloy Standard

Reference Materials. SRM 2175 MP35N Refractory Alloy had a measured phosphorus mass fraction of $6.40 \text{ mg/kg} \pm 0.56 \text{ mg/kg}$, and SRM 861 Nickel-based Superalloy with phosphorus measured at $12.71 \text{ mg/kg} \pm 0.71 \text{ mg/kg}$.

4) Sulfur in SRM 861 Nickel-based Superalloy. The U.S. aerospace industry is a leading technology driver in R&D spending and the nation's leading net exporter of manufactured products. The aircraft industry and supporting foundries and their Consortium on Casting of Aerospace Alloys brought to NIST the need of the industry to produce turbine blades with sulfur concentrations near 1 mg/kg , a critical level between acceptable and unacceptable materials. The ACD has investigated and applied its primary isotope dilution mass spectrometric method to the low concentration required, and certified sulfur at $0.561 \text{ mg/kg} \pm 0.078 \text{ mg/kg}$, almost an order of magnitude lower than any previous sulfur in metal standard. The limiting source of uncertainty in this measurement was the blank.

5) Nitrogen in SRM 1755 Nitrogen in Low Alloy Steel: Nitrogen is an important element that is monitored in the steel manufacturing process as it contributes to the hardness of the resulting steel. A high nitrogen content makes steel unsuitable for many applications. Nitrogen is typically determined in industry by matrix-dependent techniques that require calibrating standards. To produce standards requires an absolute technique for nitrogen. ACD applied the classical Kjeldahl method to the determination of nitrogen in this standard, and certified the nitrogen amount content by combining the results using this method with independent results from cooperating laboratories—vacuum gas fusion with volume measurement and combustion with infrared detection. The availability of the SRM will allow steel manufacturers to calibrate their optical emission spectrometers (OES) on the production floor and monitor the nitrogen levels during the manufacturing process. Nitrogen is certified at $118.4 \text{ mg/kg} \pm 1.8 \text{ mg/kg}$.

Metal SRMs Certified in FY 2001:

SRMs 1775/2175	MP35N Refractory Alloy	10/10/2000
SRM 1755	Nitrogen in Low Alloy Steel	12/8/2000
SRM 861	Nickel-based Superalloy	3/28/2001
SRM 131g	Low Alloy Silicon Steel	5/20/2001
SRMs 361/661	AISI 4340 Steel	5/29/2001(revision)
SRMs 363/663	Chromium Vanadium Steel	5/29/2001(revision)

Cements:

The cement industry is a significant component of the U.S. economy, with annual shipments in excess of \$4 billion. This industry is regional in nature. Because of the cost of shipping cement quickly overtakes its value, customers traditionally purchase cement from local sources. In the U.S. 50 companies operate 120 cement plants in 38 states. The vast majority of cement produced in the United States is shipped less than 500 kilometers. The dispersed nature of the cement industry, coupled with the vast quantities used in commerce, results in extremely large numbers of cement analyses made by analytical service laboratories. The cement industry uses X-ray Fluorescence (XRF), a rapid and cost effective method of analysis. XRF is effective as long as there are primary reference materials for calibration. NIST cement SRMs are the primary reference materials used throughout the U.S. and the world, and are the basis of all of the standard methods used by U.S. industry.

CSTL initiated a plan in 1998 to address the recertification and perpetuation of cement SRMs. This plan includes certification measurements made at NIST by XRF, but uses the Portland Cement

Association's premier laboratory to perform the bulk of the certification work. The matrix independent XRF technique as described above for metals has been successfully applied in the NIST certification effort.

Cement SRMs Certified in FY 2001:

SRM 1884a	Portland Cement	7/13/2001
SRM 1885a	Portland Cement	7/13/2001
SRM 1887a	Portland Cement	8/9/2001

Fossil Fuels:

1) Certification of Mercury in SRM 1632c Trace Elements in Coal: NIST has had to respond quickly to the U.S. Environmental Protection Agency's very rapid move to regulate the emission of mercury by coal-fired electric power plants. On December 14, 2000, EPA announced its intention to regulate utility mercury emissions and force reductions by coal-fired power plants.

Obviously, accurate and reliable coal analysis results are required to make meaningful estimates of mercury emissions. The Electric Power Research Institute, questioning the adequacy of the test methods that would be used by the numerous commercial and utility laboratories responding to the need to measure Hg emissions, initiated a round robin study to evaluate the adequacy of the test methods. The objective of the study was to compare the performance of the various test methods against recognized benchmarks of laboratory performance. This study was done in cooperation and collaboration with the ASTM D5 committee. Several new test methods developed by ASTM were also evaluated. The lack of coal SRMs certified for mercury content was of immediate concern to the D5 committee, EPRI, and the utilities at the start of this time period.

Two method development research efforts were pursued in parallel in ACD to improve the accuracy for mercury measurement. A new method based on isotope dilution, cold vapor, inductively coupled plasma mass spectrometry (ID-CV-ICP-MS) and an updated method based on radiochemical neutron activation analysis (RNAA) minimized previous sources of measurement uncertainty. Their analyses of SRM 1630a resulted in a five-fold reduction in the uncertainty and the certification of Hg content ($93.8 \text{ ng/g} \pm 3.7 \text{ ng/g}$). The certification of SRM 1630a was timely, as it allowed the evaluation of bias of methods used in the EPRI round robin study. Neither of the two main ASTM methods, Bomb Combustion CVAA and Acid Extraction CVAA showed significant bias, although the interlaboratory variability was very high (30 % and 20 %, respectively).

2) Development and Application of CV-ICP-MS for Mercury Certification: The CV-ICP-MS method is based on isotope dilution mass spectrometry (IDMS), combined with a sample pretreatment step and a matrix separation step to form a unique measurement process. There are two parts to the method, both of which are necessary for accurate results:

- The sample is combusted at high temperature in a closed system (Carius tube), which ensures equilibration of the natural Hg atoms with those in the enriched spike. The sample is placed in the Carius tube with a known amount of a ^{201}Hg enriched isotope, and high-purity nitric acid. The tube is flame-sealed, and heated to 240°C in a laboratory oven for approximately 6 hours. This is about 100 degrees higher than conventional microwave digestion systems. This process completely oxidizes the coal to CO_2 , and equilibrates the enriched ^{201}Hg atoms with the Hg atoms in the sample. At this

point the $^{201}\text{Hg}/^{202}\text{Hg}$ ratio is fixed and needs only to be measured accurately to determine the mercury in the original sample. Subsequent loss of sample does not affect the final accuracy. After combustion, the Carius tube is opened and the sample simply diluted with high-purity distilled water.

- Accurate measurement is made of the perturbed ratio using an ICP-MS instrument. The sample is mixed with tin (II) chloride, which reduces the oxidized mercury to volatile mercury vapor. The Hg is separated from the matrix using a cold-vapor matrix separation cell, and transferred to the ICP-MS instrument using argon carrier gas. The $^{201}\text{Hg}/^{202}\text{Hg}$ ratio is measured using conventional ion counting techniques.

The method is now considered by NIST to be a primary method and has been applied to the certification of mercury in the existing suite of coal SRMs. This suite represents a number of types of coal (chosen principally for the range of sulfur content). The mercury content certified in this suite is from 11 to 146 ng.

Coal SRMs certified for Mercury in FY2001

SRM 1632c	Trace Elements in Coal	1/11/01
SRM 1635	Trace Elements in Coal	1/4/01
SRM 2682b	Sulfur and Mercury in Coal	2/6/01
SRM 2683b	Sulfur and Mercury in Coal	1/23/01
SRM 2684b	Sulfur and Mercury in Coal	2/6/01
SRM 2685b	Sulfur and Mercury in Coal	2/6/01
SRM 2692b	Sulfur and Mercury in Coal	2/6/01

3) Determination of Sulfur in Diesel Fuels and Gasoline: The primary method for sulfur determination is based on isotope dilution thermal ionization mass spectrometry (ID-TIMS) and has been used to certify over 50 SRMs since its development in 1984. The fossil fuel sulfur SRMs are in extremely high demand because sulfur content affects their quality and price. In addition, new regulatory requirements for lower sulfur emissions have resulted in the demand and development for new materials. The analytical work in FY 2001 reflects the need to meet these new regulatory requirements. SRM 1620c Sulfur in Residual Fuel Oil 4 % was certified to provide a high-level sulfur standard for the electric power industry. SRMs 2298 and 2299 Sulfur in Gasolines were analyzed to benchmark new low-level S in gas regulations. And, BCR 104R, BCR 671, and BCR 672 were three new reference materials (CRMs) of the European Union's Community Bureau of Reference (BCR). NIST collaborated with the Institute for Reference Materials and Measurements (IRMM) in the certification process of these CRMs. This collaborative work between National Measurement Institutes reflects the need to provide comparability and traceability for measurements and standards on a global basis.

New EPA regulations go into effect in 2007 that will limit sulfur in gasoline to 30 mg/kg (ppm). SRMs 2298 and 2299 are new low-level sulfur in gasolines that anticipate these regulations and will allow the development of new methods and instrumentation to make measurements at this level. With indicated concentrations at 4.5 and 13.6 mg/kg, respectively, these measurements were especially challenging—sample sizes had to be minimized to achieve controlled digestion and blanks at the ppm level are significant. Nonetheless, these standards should meet the more stringent analytical requirements. The development of homogeneous materials for sulfur at such low levels and reducing sulfur contamination in the measurement process are challenges to be faced in the future.

The BCR CRM “gas oils” are heating oil and two diesel fuels; they have indicated sulfur levels between 0.02 % to 0.10 %. These standards anticipate the EEC regulations that limit the maximum sulfur in gasoil to 0.10 % by the year 2008. Former BCR gasoil standards were certified by interlaboratory exercises. However, the IRMM proposed to certify these standards by methods more traceable to the SI and, consequently, they developed the NIST ID-TIMS procedure. (The one difference, however, was the use of closed microwave dissolution instead of NIST’s Carius tube dissolution practice). Since this method was new and unproven at IRMM, NIST collaborated in the certification process, which is scheduled to be completed in Spring 2002. Two of these standards were also distributed in a Pilot Study of CCQM (P-26 Sulfur in Fuel).

Fossil Fuel SRMs Certified for Sulfur in FY 2001

SRM1620c Sulfur in Residual Fuel Oil 4 % 3/22/01

Titanium Dioxide:

Assay of Titanium Dioxide by Coulometric Back-Titration: Titanium dioxide is a commodity that is basic to the paint and paper industries, as well as a major component in many ceramic materials. SRM 154 Titanium Dioxide is the benchmark for purity measurement and an accurate assay value is essential for product quality control in these industrial sectors. In the re-certification of SRM 154c Titanium Dioxide, the target uncertainty for the certified value was less than 0.1%. A coulometric procedure of primary nature was developed and applied; the total uncertainty achieved was 0.062 %.

Titanium dioxide assay techniques rely on the oxidation-reduction chemistry of titanium (Ti). In titanium dioxide, Ti is present as 4-valent Ti(IV). Following dissolution, this Ti(IV) can be reduced in a Jones reductor to 3-valent Ti(III). To obtain the assay value, this Ti(III) must be quantitatively reoxidized to Ti(IV) adding excess oxidizing agent which itself has an accurately-known assay value. It is also necessary to establish that the reduction of the Ti(IV) to Ti(III) is quantitative. In addition, it is necessary to take into account any impurities in the titanium dioxide that react with the oxidizing agent in a similar manner to Ti(III) and to make corrections for these systematic sources of bias. The final certified uncertainty must include the contributions from all component uncertainties arising from each of these possible sources.

The analytical approach used a dissolution procedure adopted from state-of-the-art industrial practice, followed by reduction of Ti(IV) in the resulting solution to Ti(III) by a Jones reductor. The reduced Ti was then reoxidized to Ti(IV) by addition of an accurately known amount of the solid potassium dichromate ($K_2Cr_2O_7$), NIST SRM 136e, whose assay was determined by coulometry at NIST. The oxidizing agent was added in solid form to avoid any uncertainty from solution handling and evaporation. The amount of potassium dichromate added was greater than that required to oxidize the Ti(III) present and, hence, excess potassium dichromate remained after all the Ti was reoxidized back to Ti(IV). The excess potassium dichromate remaining in the solution was then determined by a separate coulometric titration identical to the assay procedure for the SRM 136e. The difference corresponded to the Ti(IV) present.

The coulometry used for both these titrations is an absolute method (i.e. one not requiring calibration with a chemical standard). Hence, the final assay value for SRM 154c is not dependent on another chemical standard, but rather it is based on fundamental laws (Faraday's Laws of Electrolysis) and a fundamental constant (the Faraday constant). Efficiency of the Jones reductor was assessed by analysis of Ti metal of known purity. The reduction of the dissolved Ti solution

and the subsequent titrations were procedurally identical to the corresponding titanium dioxide assays.

A glow-discharge mass spectrometric analysis of the new SRM 154c was performed to account for possible interfering elements. This analysis yielded the trace contents of virtually all elements in the periodic table. Corrections were made to account for the known oxidation-reduction chemistry of those elements that interfere with the titanium dioxide assay. The final certified value for SRM 154c is 99.591 % with an uncertainty of 0.062 %. This uncertainty includes the component uncertainties from each of the sources discussed above and, in contrast to the previous certification, the present result conforms to the revised NIST policy on the treatment of uncertainty.

Titanium Dioxide SRM certified in FY 2001

SRM 154c Titanium Dioxide 6/1/01

Impact: In February 2000, the Economic Impact of SRMs for Sulfur in Fossil Fuels was published that quantified a portion of the economic benefits associated with sulfur SRMs, which were tremendous. The conclusions of this study can be generalized to other commodities as well. The development of methods and certification of SRMs result from NIST's unique expertise in measurement. SRMs play a key role in the National Measurement System for analytical chemistry. They serve as national primary standards and are used as calibrants and as quality assurance materials to evaluate measurement accuracy, to intercalibrate laboratories in measurement programs, and to provide compatibility of measurement data. NIST SRMs greatly improve measurement accuracy by reducing the uncertainty of measurement. These improvements enhance the products and services of the measurement industry, such as testing laboratories and analytical instrument manufacturers. More accurate elemental content information also reduces the likelihood of disputes between buyers and sellers.

Future Plans: The ACD will continue the difficult task of balancing the need to develop and benchmark new measurement capabilities, while at the same time maintaining core capabilities. The ACD will continue to address industry's need to have the best calibration materials so that they can make fast, inexpensive, and accurate measurements. We will address this need not only by the development of new reference materials, but also by the development of new instrumental matrix-independent and high performance methods that should reduce the dependence upon these matrix-matched reference materials.

Methods and Standards for Advanced Materials Characterization

CSTL Program: Chemical Characterization of Materials

Authors: *G.P. Lamaze, H. H. Chen-Mayer, R.L. Paul, and M. Salit*

Abstract: The Analytical Chemistry Division maintains an active program of advanced materials characterization through the application of a wide variety of measurement techniques. These techniques are used to determine elemental concentrations in SRMs for producers of advanced materials, as well as standards for calibration of surface and other analytical techniques. This year we have developed a radiochemical procedure to determine the amount of ion-implanted phosphorus in a silicon wafer by neutron activation analysis. This method will be used in conjunction with Division 837 to certify an SRM to meet a high-priority request of the semiconductor industry. We have also been working with EEEL and Division 837 to characterize sub-samples of epitaxial thin-film prepared AlGaAs. The remaining portions of which would then be used as primary standards to calibrate non-destructive probe techniques, ultimately in the preparation of a new suite of SRMs for Al:Ga ratios in AlGaAs. The Al:Ga substitution ratio is critical for the optoelectrical properties of devices fabricated in thin films of AlGaAs. Non-destructive probe techniques, such as x-ray fluorescence, while precise, have been demonstrated to show a bias in these ratios.

In addition, ACD maintains and operates two cold neutron beam facilities and have access to a large number of other neutron techniques through our collaborations with the Center for Neutron Research. Customers for these services include industry, universities and other government organizations. Proprietary research is on a cost recovery basis. During the past year, we have made a number of measurements that have helped solve problems in the areas of semiconductor technology and thin film science. One example of semiconductor research is a study of nitrogen content in GaN/GaAs bilayers with Corning. The material system GaN/AlGaAs holds promise for the production of blue light emitting lasers. The N concentration is a crucial parameter for establishing the device characteristics. SIMS can measure the N profile of the grown GaAsN layers if provided with a calibrated GaAs/GaN standard. Corning scientists have come to us for quantitative nitrogen determination in a GaN/GaAs system and to establish an in-house calibration capability. Another example is the correlation of manufacturing conditions with the lithium distribution of lithium phospho-oxynitride layers used in lithium batteries. This is being done in conjunction with Tufts University as part of a long-term research program in battery materials.

Purpose: The purpose of our efforts is to transfer NIST accuracy and to industry, academia and other government agencies. In addition, we provide our measurement capabilities directly to industry to solve important measurement problems when the required capabilities do not exist outside of NIST. The cold neutron instruments at NIST are operated as part of a national user facility. These instruments have been established with the intent of furnishing the US scientific community (industry, academia, and government) with state of the art facilities for research with neutrons. Our purpose is to maintain the facilities, make measurements and perform data analysis in order to improve measurement capabilities. The two facilities operated by the ACD (NDP and PGAA) cover a wide range of applications with semiconductor materials making up a large part of the materials analyzed. The neutron depth profiling instrument, by its nature, makes measurements only on thin films and surfaces (up to about 10 μm), while the PGAA instrument measures elemental concentrations in bulk samples.

Major Accomplishments:

- Developed a radiochemical activation analysis procedure to certify phosphorus in implanted silicon. See Figure 1.
- Developed a digestion procedure for epitaxial thin-film prepared AlGaAs, and established that the analytical scheme would have sufficient signal-to-noise ratio to perform the requested measurements with uncertainties in the Al:Ga ratio of less than 1% relative
- Studied of GaN/GaAs bilayers (Corning). See Figure 2.
- Evaluated lithium depletion in LiNbO₃ (Corning).
- Determined lithium in thin films for battery applications (Tufts University). See Figure 3.
- Studied neutron induced soft upsets in semiconductor devices (Texas Instruments)
- Evaluated boron mobility in ion implanted boron in Tungsten (Aberdeen Proving Grounds and USMA).
- Characterized thin films using Neutron Depth Profiling and Neutron Reflectometry (with Center for Neutron Research). See Figure 4.
- Assessed long term stability of primary boron and lithium thin film standards (with Physics Lab).

Impact: Our measurement capabilities are unique in the US and are not available as in-house capabilities for individual industries. We provide a series of tools to allow industrial researchers to calibrate, understand, and evaluate production processes. Because our work is both quantitative and of high accuracy, our customers use measurements made at NIST to calibrate their own instruments. This is especially true of NDP measurements that are used to calibrate SIMS instruments.

Future Plans: The emphasis on semiconductor materials and thin films continues. The new cold source now being installed at the NIST reactor will approximately double the effective neutron flux. In the near future (FY04?), both the NDP and PGAA instruments will be moved to a new curved neutron guide that will greatly improve the signal-to-noise of both instruments. We continue to invest in improvements to both instruments so future customers will have the best available resources for their experiments. We will also perform basic research, such as stopping power studies of charged particles to provide more accurate parameters for NDP analysis.

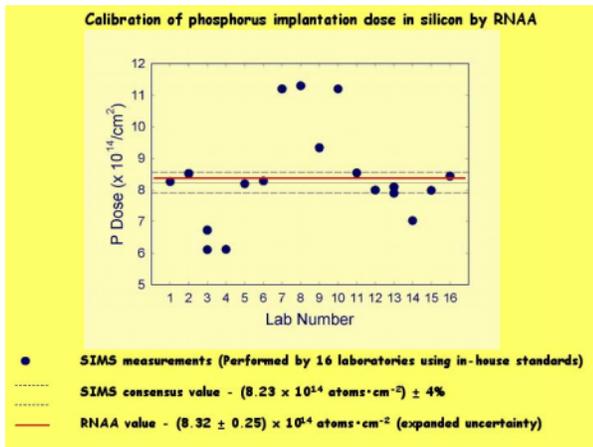


Figure 1. A new phosphorus implant in silicon SRM, certified by radiochemical neutron activation analysis (RNAA), is in development. An RNAA procedure has been developed and tested through analysis of 12 samples of silicon implanted at a nominal dose of $8.5 \times 10^{14} \text{ atoms cm}^{-2}$. A mean and standard deviation of $(8.32 \pm 0.11) \times 10^{14} \text{ atoms cm}^{-2}$ (relative standard deviation = 1.3 %) was obtained, with an expanded uncertainty of about 3 %. A comparison with SIMS measurements is shown.

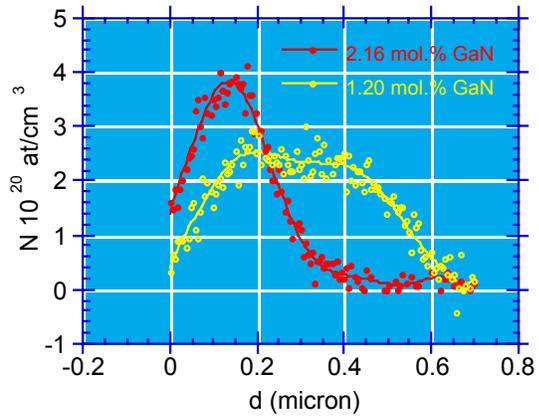


Figure 2. Distribution of N in the GaAs matrix. Both samples were about 10% thicker than expected giving important feedback to the manufacturing process.

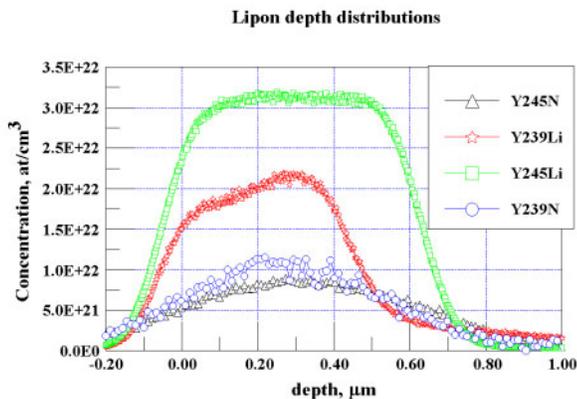


Figure 3. Depth profiles of lithium and nitrogen for two different lithium phospho-oxynitride samples. Results show that sample Y245 has the more desirable lithium to nitrogen ratio.

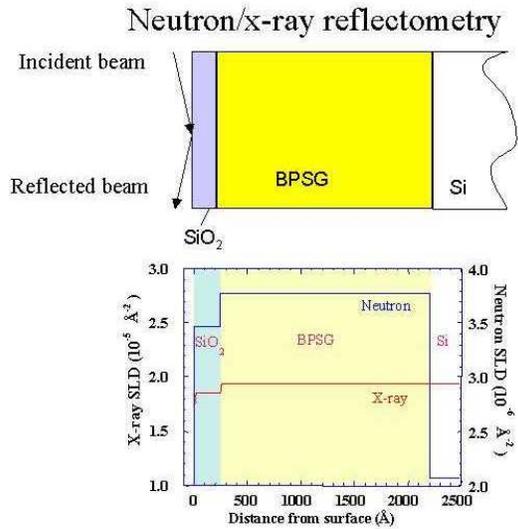


Figure 4. Schematic showing experimental reflectometry arrangement and determined density variations. When combined with NDP spectra, depth scaled is determined unambiguously.

The Investigation of Green Chemical Cleaning Processes

CSTL Program: Chemical Characterization of Materials

Authors: *L.L. Yu; and C.S. Grant (North Carolina State University)*

Abstract: The use of calcium ion sequestrants provides an environmentally benign alternative to strong acids for the cleaning of mineral deposits, typically calcium phosphate, calcium oxalate and calcium carbonate, on stainless steel surfaces, which is the current industrial practice. Basic research to characterize the mechanism and kinetics of this green chemical cleaning process has been initiated at North Carolina State University (NCSU). The focus of the current work is on the use of sodium polyaspartate (pAsp), because it is non-toxic and biodegradable in the range of molecular weights (~10,000) considered. The research requires the identification and quantification of calcium and its species on-line under various mechanical cleaning conditions; however, the measurement of these species is a major challenge. NIST and NCSU have collaborated in the development of techniques for calcium species quantification. A tandem technique has been developed that incorporates size-exclusion chromatographic (SEC) separation of the species, spectrophotometric (UV/VIS) determination of pAsp, and inductively coupled plasma optical emission spectrometric (ICPOES) determination of each calcium species. Free calcium ions and calcium-pAsp complex species have been determined. The next stage in this collaboration is the transfer of the technique to NCSU to study the mechanism and kinetics of the cleaning process during various test conditions.

Purpose: The purpose of this research collaboration is to apply the unique chemical measurement technology and expertise that resides at NIST to a problem with both major industrial and environmental consequences. This green chemical cleaning process addresses the industrial problem of scale formation, which can result in the serious loss of heat exchange efficiency and the obstruction of fluid flow. It is an especially important problem for the chemical industry.

Major Accomplishment: We have studied the properties of calcium and its pAsp complex and have developed SEC-UV/VIS-ICPOES tandem technique for the on-line measurement of calcium and its pAsp species.

Impact: The development of environmentally friendly cleaning processes will save industry money while at the same time reducing the need for chemical disposal/treatment.

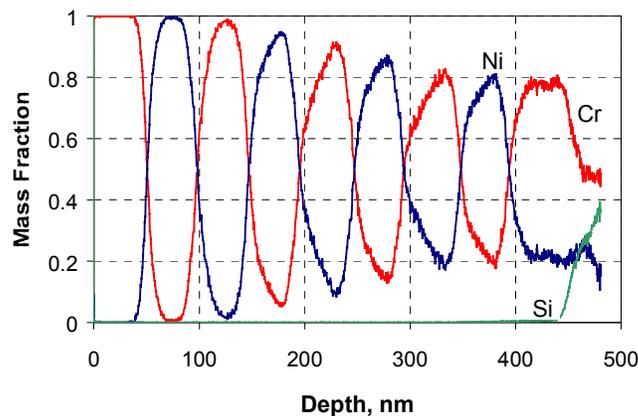
Future Plans: The on-line measurement technique is being implemented at NCSU for on-line monitoring of the calcium species in the study of cleaning mechanisms.

Studies of Quantitative Depth Profiling Using Glow Discharge Optical Emission Spectrometry

CSTL Program: Chemical Characterization of Materials

Authors: *M.R. Winchester; and D.S. Simons (837)*

Abstract: The quantitative determination of elemental composition as a function of depth on surfaces is an important analytical problem in a wide variety of modern technologies. Examples include the characterization of galvanneal layers on steel, the measurement of the composition and thicknesses of paint layers on various substrates, and the evaluation of the depth distributions of dopants in Si wafers. While the quantitative determination of elemental composition as a function of depth is likely to become even more important in the future, this remains a difficult, time consuming, and expensive metrological problem. Consequently, there is a need for better, faster, and more economical characterization methods. At NIST, glow discharge optical emission spectrometry (GDOES) is being developed and critically evaluated for this purpose. GDOES is inherently much faster and much less expensive than most competing methods of surface analysis. The current major thrust of the research is on evaluating and improving the quality of the quantitative depth profiling (QDP) data provided through GDOES. A GDOES QDP method for SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard) is being developed and the GDOES QDP results can eventually be compared to existing AES and SIMS data for this material. When fully developed, GDOES QDP may provide United States industry with the ability to make these important measurements more rapidly and less expensively than is currently possible. These developments may be expected to affect industry sectors that rely upon thin films, coatings, and other types of surface layers, such as the microelectronics and advanced materials industries.



Purpose: The quantitative determination of elemental composition as a function of depth on surfaces is an important analytical problem in a wide variety of modern technologies. Examples include the characterization of galvanneal layers on steel, the measurement of the composition and thicknesses of paint layers on various substrates, and the evaluation of the depth distributions of dopants in Si wafers. While the quantitative determination of elemental composition as a function of depth is likely to become even more important in the future, this remains a difficult, time consuming, and expensive metrological problem. Consequently, there is a need for better, faster, and more economical characterization methods. At NIST, glow discharge optical emission spectrometry (GDOES) is being developed for this purpose. GDOES is inherently much faster and

much less expensive than most competing methods of surface analysis. A major thrust of the research is on evaluating and improving the quality of the quantitative depth profiling (QDP) data provided through GDOES.

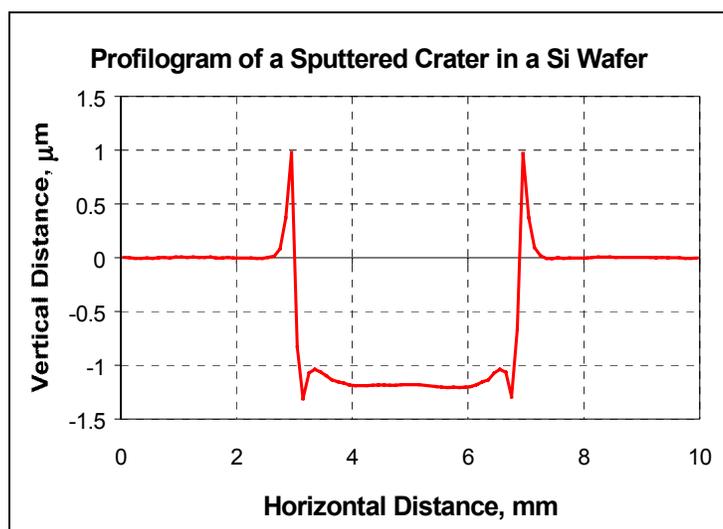
Major Accomplishments: In ongoing work begun during fiscal year 2001, a GDOES QDP method for SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard) is being developed. This sample consists of nine alternating layers of Ni and Cr, each approximately 55 nm thick, on a Si wafer. SRM 2135c has previously been examined in detail using Auger Electron Spectroscopy (AES) and Secondary Ion Mass Spectrometry (SIMS). Therefore, GDOES QDP results can eventually be compared to existing AES and SIMS data.

The first quantitative depth profile (i.e., mass fraction vs. depth) obtained for SRM 2135c is shown in the figure. These data were acquired at glow discharge conditions of 700 V and 20 mA using the SRM 1760 Low Alloy Steel series for calibration. One of the strengths of GDOES QDP is that calibration may be accomplished using bulk calibrants only. Referring to the figure, note that while the depth resolution decreases somewhat with increasing depth, the thicknesses of the Ni and Cr layers are approximately correct. Since these data were acquired, alternative discharge conditions that improve depth resolution significantly have been found. However, calibration has not been performed at these new conditions, and so quantitative data are not yet available. One observation that has been made during this work is that the walls of the craters formed during glow discharge sputtering may sometimes be sloped significantly, even when the crater bottoms are very flat. Upon further study, this may prove to be an important point, because common calibration algorithms assume vertical crater walls.

Impact: The NIST effort to develop GDOES for the quantitative determination of elemental composition as a function of surface depth is just beginning. When fully developed, GDOES QDP may provide United States industry with the ability to make these important measurements more rapidly and less expensively than is currently possible. These developments may be expected to affect industry sectors that rely upon thin films, coatings, and other types of surface layers, such as the microelectronics and advanced materials industries.

Future Plans: GDOES QDP research at NIST will likely continue for at least several more years. Specifically, during fiscal year 2002 the method for SRM 2135c will be refined and evaluated further, including comparisons of data with existing AES and SIMS data. Methods for other important sample types, such as Si wafers implanted with B and other dopants, will also be developed and evaluated. Finally, the effects of sloping crater walls on calibration will be investigated further.

M.R. Winchester (839) and D.S. Simons (837) Glow discharge optical emission spectrometry (GDOES) is being developed for the quantitative determination of the elemental composition of surfaces as a function of depth. GDOES is inherently much faster and less expensive than most competing methods of



surface analysis. A major thrust of the NIST research is on evaluating and improving the quality of data. The capabilities of GDOES quantitative depth profiling are demonstrated through the development of a GDOES method for the characterization of SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard). This sample consists of nine alternating layers of Ni and Cr, each layer being approximately 55 nm thick, on a Si wafer. While the development of the GDOES method for this sample is still underway, the preliminary results are encouraging. All nine alternating layers have been resolved, and the layer thicknesses determined using GDOES are approximately correct. The NIST effort to develop GDOES as a tool for quantitative depth profiling of surfaces will likely continue for at least several more years. Once fully developed, GDOES should provide United States industry with a useful metrological tool, potentially affecting many industries that rely upon thin films, coatings, and other surface layers.

Research in Chiral Separations

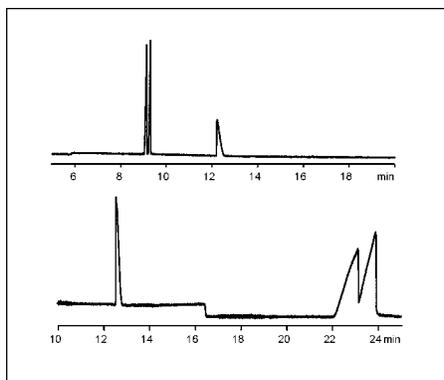
CSTL Programs: Chemical Characterization of Materials, Healthcare Measurements

Author: *K.W. Phinney*

Abstract: A multi-faceted research program has been conducted to gain a better fundamental understanding of chiral separation mechanisms and to address some of the current measurement challenges. Although much of the work in this area has focused on pharmaceutical compounds (drugs), the work also has implications for other areas, including agricultural, food, and forensic sciences. This program encompasses a variety of analytical techniques including gas, liquid, and supercritical fluid chromatography, as well as capillary electrophoresis. The enantioselectivity of several sulfated cyclodextrins was investigated as part of an ongoing effort utilizing capillary electrophoresis to study chiral recognition processes. The use of well-characterized chiral selectors is necessary for reliable and reproducible chiral analysis. A liquid chromatographic method for the separation of amphetamine and methamphetamine enantiomers isolated from hair samples was developed. Determination of enantiomeric composition of these drugs is essential for distinguishing drug abuse from legitimate drug use. An SRM designed to evaluate the performance of chiral stationary phases used in liquid chromatography was also introduced during the past year. This SRM can be used to monitor changes in column performance over time and for control of column manufacturing processes.

Purpose: Enantioselective separations have grown in importance in several industries, but the greatest demand has been in the pharmaceutical industry. Chiral drugs now represent a \$100 billion market, and measurements are needed to meet regulatory requirements associated with their development and production. Chiral separations also play a role in agrochemicals, foods and beverages, and forensic science. No one analytical technique can meet all the needs of these diverse fields. Therefore, our research has focused on various types of analytical methodology including gas, liquid, and supercritical fluid chromatography, as well as capillary electrophoresis. Development of rugged and accurate chiral analysis methods is facilitated by an understanding of chiral recognition processes and by the use of chiral additives or chiral stationary phases having consistent properties and compositions.

Major Accomplishments: The enantioselectivity of several commercially available sulfated cyclodextrins was investigated by utilizing them as chiral additives in capillary electrophoresis. In the forensic area, the use of liquid chromatographic methodology for the separation and quantification of amphetamine and methamphetamine enantiomers isolated from hair samples was investigated. An SRM designed to monitor the performance of chiral stationary phases for liquid chromatography was also introduced within the past year.



Separation of chlorpheniramine maleate enantiomers with two different sulfated cyclodextrins.

Impact: Some of the methodology developed at NIST has been implemented in the pharmaceutical industry, and our research efforts have provided much needed information to guide method development efforts in a variety of fields. We also anticipate that this work will ultimately lead to the availability of improved separation technologies.

Future plans: The development of additional SRMs to validate enantiomeric composition measurements is currently being pursued, with an emphasis on chiral drugs of abuse. Efforts to characterize and improve chiral stationary phase performance will continue in conjunction with investigations of chiral recognition mechanisms.

Fourier-Transform Microwave Spectroscopy for Quantitative Measurements

CSTL Programs: Chemical Characterization of Materials, Environmental Measurements

Authors: *R.S. DaBell, and P.M. Chu; and R.D. Suenram (844)*

Abstract: There is a continuing need for improved analytical techniques to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change. The use of Fourier-transform microwave (FTMW) spectroscopy as a quantitative analytical technique is being investigated as its high spectral resolution and high sensitivity spectroscopy suggest that it can provide near real-time response and unambiguous identification of analytes in a gas sample. After incorporating instrument optimizations and modifications in response to initial evaluation studies, NIST measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices. As work to optimize the instrument performance continues, an effort will also focus on evaluating the capability of FTMW spectroscopy to measure analytes in gas matrices that more closely match ambient air.

Purpose: To establish the feasibility of Fourier-transform microwave (FTMW) spectroscopy as a reliable technique for quantifying trace-gas analytes.

Major Accomplishments: There is a continuing need for improved analytical techniques to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change. Towards this end, the use of Fourier-transform microwave (FTMW) spectroscopy as a quantitative analytical technique is being investigated. The high spectral resolution and high sensitivity of FTMW spectroscopy suggest that the technique can provide near real-time response and unambiguous identification of analytes in a gas sample. The principal goal of the present program is to evaluate the technique's potential as a reliable and robust tool for quantitative measurements of trace gases in ambient air samples. To achieve these goals requires: 1) evaluation of the instrument function; 2) consideration of sample delivery issues; 3) comparison with other established analytical techniques; and 4) blind sample testing.

Initial evaluation of the instrument revealed that measured peak positions for a given analyte were reproducible to within 5 kHz providing powerful and reliable information for species identification. The signal intensities, however, varied substantially over long measurement periods (12 h), with worst-case scenarios showing complete loss of signal. To regain signal intensity, it was necessary to adjust or modify a number of instrument parameters. To improve the reliability of signal intensity, the performance of each individual component was evaluated and several instrument modifications have been incorporated. Currently, the remaining drift is $\approx 10\%$ over 12 h and the chief source of drift has been traced to fluctuations in the resonant frequency of the Fabry-Perot cavity caused by temperature induced changes of the optical cavity length. Concomitant with our effort to improve the instrument performance, we have also undertaken preliminary tests to examine the signal intensity as a function of the sample

concentration and determine the detection limits using NIST standard reference samples of sulfur dioxide in a nitrogen matrix and ethanol in a blended air matrix. For sulfur dioxide, using a sample concentration range of 50 $\mu\text{mol/mol}$ to 1000 $\mu\text{mol/mol}$, a 0.14 $\mu\text{mol/mol}$ detection limit was obtained. For ethanol, using a concentration range of 200 $\mu\text{mol/mol}$ to 1400 $\mu\text{mol/mol}$, the detection limit was 1.3 $\mu\text{mol/mol}$. In both cases, the signal intensity was linear with the sample concentration over the entire ranges. These measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices.

Publications: DaBell, R. S., Chu, P. M., Fraser, G. T., Suenram, R. D., “Evaluation of Fourier-transform microwave spectroscopy as a tool for quantitative analysis: Signal stability considerations”, Proc. SPIE, 4574, to be published (2001).

Impact: After incorporating instrument optimizations and modifications in response to initial evaluation studies, NIST measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices. Next steps will focus on evaluating its capability to measure multiple analytes in gas matrices that more closely match ambient air. Improved, validated analytical techniques are needed to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change.

Future Plans: As work to optimize the instrument performance continues, an effort will also focus on evaluating the capability of FTMW spectroscopy to measure analytes in gas matrices that more closely match ambient air. Since this technique is based on probing rotational transitions and requires a supersonic expansion of the gas sample to concentrate the population distribution, different sample matrices will strongly impact the properties of the expansion and ultimately affect the detection limits. To date, quantitative measurements have not been the major driving force of FTMW studies and most measurements are made using an idealized balance gas of helium and neon. An effort will be focused on characterizing the signal intensities for samples with high concentrations of nitrogen, oxygen, carbon dioxide, and water, which are at high concentrations in analytically relevant samples.

Quantitative Infrared Database Developed to Support Remote Sensing Applications

CSTL Program: Environmental Measurements

Authors: *P.M. Chu, F.R. Guenther, G.C. Rhoderick, and P.A. Johnson*

Abstract: Over the last decade, growing concerns about the environment in general and air quality in particular have stimulated the development of improved, cost-effective field monitoring methods. With the recent advances in optical-based technologies, methods such as open-path Fourier transform infrared (FT-IR) spectroscopy are now becoming viable tools for field measurements. The *in-situ* and real-time nature of this approach offers several advantages over traditional point source monitoring techniques. Since molar absorptivity data in the literature widely differ, a validated quantitative database traceable to national measurement standards is a critical part of the infrastructure required for establishing emerging infrared-based monitoring technologies. NIST developed Standard Reference Database 79 (SRD 79) “Quantitative Infrared Database” to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP). SRD 79 data are based on NIST primary gravimetric standards prepared with starting materials of assessed purity and procedures that minimize contamination. Currently, absorption coefficient data for 40 HAPs on a US EPA priority list is available as SRD 79, Version 1.1 with Data Release 3.0. Additional spectra will be added to the database as they are acquired and updates will be available over the internet. Plans include continued data acquisition for the compounds listed in the 1990 US EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading.

Purpose: To develop the NIST Standard Reference Database 79 (SRD 79) “Quantitative Infrared Database” to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP).

Over the last decade, growing concerns about the environment in general and air quality in particular have stimulated the development of improved, cost-effective field monitoring methods. With the recent advances in optical-based technologies, methods such as open-path Fourier transform infrared (FT-IR) spectroscopy are now becoming viable tools for field measurements. The *in-situ* and real-time nature of this approach offers several advantages over traditional point source monitoring techniques for applications such as determining fugitive emissions and chemical contaminants from industrial processing plants, hazardous waste and municipal landfills, water treatment plants, oil refineries, and chemical plants. Following successful testing of FT infrared methods during remediation of several Superfund sites, the US Environmental Protection Agency (EPA) has issued a protocol (TO-16) for FT infrared open-path remote sensing. Since molar absorptivity data in the literature widely differ, a validated quantitative database traceable to national measurement standards is a critical part of the infrastructure required for establishing emerging infrared-based monitoring technologies. New infrared-based technologies coupled with the NIST spectral database provides both industry and EPA with a tool for assessing regulatory compliance that is both cost-effective and less invasive.

SRD 79 data are based on NIST primary gravimetric standards prepared with starting materials of assessed purity and procedures that minimize contamination. For each compound, the absorption coefficient spectrum was calculated using nine transmittance spectra and the Beer's law relationship. The uncertainties in the absorption coefficient data are estimated from the data analysis and considerations of other error sources such as the non-linear detector response.

The primary effort of this project has focused on acquiring data at 25 °C. To address the data needs of communities measuring industrial stack emissions by passive FT-IR and other infrared-based technologies, we have measured absorption coefficients for industrially important target gases, sulfur dioxide and methanol, at temperatures ranging from 25 °C to 200 °C. This project included studies to quantify sample emission contributions to the measured single-beam spectra.

Major Accomplishments: Currently, absorption coefficient data for 40 HAPs on a US EPA priority list is available as the NIST Standard Reference Database (SRD) 79, Version 1.1 with Data Release 3.0. The data are stored in the standard JCAMP-DX format to enable universal access to the data. The 0.12 cm⁻¹ resolution data were processed to generate data at a number of different resolutions and apodizations to provide users with data that closely match their experimental parameters. A digital signature accompanies each data file, allowing users to ensure the integrity and source of the data file and traceability to NIST.

Data comparisons with Battelle Pacific Northwest laboratories and the higher temperature data project have helped us characterize two additional FT-IR intensity artifacts. Both of these artifacts are associated with the single limiting aperture design in most commercial FT-IRs. The first artifact is attributed to the detector viewing the warm annulus of the aperture in higher resolution measurements (0.2 cm⁻¹ or higher). The off-axis rays distort the line shapes and increase the observed band intensities. The second artifact is due to the light exiting the interferometer, which is directed towards the source, can be reflected off the aperture back into the interferometer. This double modulated light produces a 2f alias throughout the spectrum. Reducing the reflectivity of the aperture wheel minimizes this artifact.

Adding an additional aperture system or a multipass cell after the interferometer minimizes the off-axis rays that reach the detector and in turn the contributions from these artifacts. For SRD 79, all of the data was acquired with a multipass cell and the potential biases due to these artifacts are within the stated uncertainties in the finger print region and no biases are expected at higher frequencies. The higher temperature absorption coefficient data required additional measurements with the infrared source off to appropriately account for sample emission issues. At 200 °C, the sample emission correction is approximately 10 % for bands in the spectral region near 1000 cm⁻¹ for the instrument and optical configuration used.

Recent Publications:

“Intensity Artifacts in Gas Phase FTIR Spectroscopy: Focus on the Aperture”, S.W. Sharpe, R.L. Sams, T.J. Johnson, and P.M. Chu, *Fourier Transform Spectroscopy, Trends in Optics and Photonics Series, Vol 51, Optical Society of America, 2001.*

“Infrared Absorptivity Temperature Dependence of Gas Phase Methanol and Sulfur Dioxide”, P.M. Chu, G.C. Rhoderick, P.A. Johnson, NISTIR 6773, National Institute of Standards and Technology, Gaithersburg, MD 20899, August 2001.

“Removing Aperture-Induced Artifacts from FTIR Intensity Values”, T.J. Johnson, R.L. Sams, T.A. Blake, S.W. Sharpe, and P.M. Chu, submitted to Applied Optics.

Data Release 3.0 for NIST Standard Reference Database 79 CD, P.M. Chu, F.R. Guenther, G.C. Rhoderick, National Institute of Standards and Technology, Gaithersburg, MD 20899-8393, August 2001.

Impact: New infrared-based technologies coupled with the NIST Standard Reference Database 79 (SRD 79) “Quantitative Infrared Database” to support the optical-based measurements of chemical emissions and hazardous air pollutants provides both industry and EPA with a tool for assessing regulatory compliance that is both cost-effective and less invasive.

Future Plans: This quantitative infrared database is an ongoing project at NIST. Additional spectra will be added to the database as they are acquired and updates will be available over the internet. Plans include continued data acquisition for the compounds listed in the 1990 US EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading. Additionally, comparisons of NIST primary standards and molar absorptivity data with National Metrology Institutes and other laboratories will be expanded to facilitate the use of this database in issues of global interest and impact.

Establishing a system for primary realization of atmospheric ozone measurements

CSTL Programs: Environmental Measurements, International Measurement Standards

Authors: *P.M. Chu, and J.E. Norris*

Abstract: Ozone, at tropospheric levels, is a health concern and contributes to climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. These concerns have led to the establishment of air quality standards and international protocols to reduce the emissions of pollutants that either contribute to tropospheric ozone formation or deplete stratospheric ozone. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest in ozone measurements has prompted eleven national laboratories to acquire NIST SRPs.

Purpose: To develop an advanced primary ozone standard with improved SI-traceability. This project is in partnership with researchers at the Bureau International des Poids et Mesures (BIPM) to assure the comparability and SI-traceability of global ozone measurements.

The concentration of ozone in the atmosphere remains a significant issue from both scientific and political perspectives. Ozone, at tropospheric levels, is a health concern and contributes to climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. These concerns have led to the establishment of air quality standards and international protocols to reduce the emissions of pollutants that either contribute to tropospheric ozone formation or deplete stratospheric ozone. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest in ozone measurements has prompted eleven national laboratories to acquire NIST SRPs.

Major Accomplishments: To support the growing national and international need of improved ambient ozone measurements, NIST is partnering with BIPM to develop an advanced primary ozone reference standard and to share the dissemination of secondary or transfer standards. The basic project plan includes 1) Transfer the current ozone “national reference photometer” technology to BIPM. 2) Assess the performance of the current technology with respect to current and future ozone measurement requirements. 3) Provide recommendations for potential improvements of the SRPs and survey other candidate secondary standards. 4) Develop a primary reference standard with capabilities of SI-traceability verification.

The major challenge of this project is to incorporate a secondary method that can provide a solid foundation for the primary reference standard at relevant ozone concentrations (0 nmol/mol to 100 nmol/mol). Additionally, any uncertainties due to ozone transport must be eliminated. Several approaches are being considered. Gas phase titration with nitric oxide is an attractive method, since the ozone concentration can be directly linked to primary gravimetric NO and NO₂ standards. Success of this project, independent of the verification method, demands

instrumentation with high sensitivity, accuracy, precision, and rapid time response for measuring chemical species at atmospheric levels (nmol/mol and lower). In fact, minimum detection sensitivities better than 1 nmol/mol will be required to maintain uncertainties at the 1 % level for the current ground level ozone concentrations. A number of in-situ techniques are being considered, including time-gated laser-induced fluorescence detection, tunable diode laser absorption spectroscopy, and cavity ring-down spectroscopy for detection of relevant chemical species and possible contaminants in the carrier gas.

Current efforts have focussed on the construction of two new NIST SRPs which are to be transferred to BIPM shortly and an initial performance evaluation of the current SRPs. During an electronics upgrade of eleven instruments, a distinct 0.5 % slope calibration bias was noted for most of these instruments compared to SRP 2, the current principal NIST reference photometer. The calibration bias may be due to: sample memory effects in SRP 2, fluctuations in both temperature and pressure measurements for a number of instruments, variations in the zero offset dependent on the sample configuration, and significant detector drifts. An ongoing effort is dedicated to identifying and eliminating this bias.

Impact: As a collaborative project between NIST and BIPM, the advanced primary ozone photometer will provide an internationally validated ozone standard that can be used to anchor all ozone measurements through a defined traceability structure. The accuracy of the NIST SRP would be checked for consistency with the NIST SRM gas program and primary standards developed by BIPM.

Future Plans: NIST collaboration with BIPM will continue with the transfer and evaluation of the two new NIST SRPs. NIST is working to identify and eliminate the measurable calibration bias as discussed above. We are also currently surveying appropriate verification methods and state-of-the-art instrumentation that can provide a solid foundation for the new primary reference standard.

High-Accuracy Coulometric Analysis of Gas Mixtures

CSTL Programs: Environmental Measurements, Chemical Characterization of Materials

Author: *K.W. Pratt*

Abstract: Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry. Standard gas mixtures of assessed composition are needed for calibration of instrumentation, method validation, and quality control purposes. The state of the art technique for generating standard gas mixtures is gravimetric preparation, in which the masses of the component gases introduced into a cylinder are measured – providing concentration data. However, for acidic gases, this approach often fails. The introduced active gas can react with the inside surface of the cylinder, changing its concentration in the mixture. Long-term storage of such mixtures is often impossible without change in the concentration of the acidic gas component and methods are needed to provide accurate measurements of these strong acid components in gas mixtures. Initial studies at NIST have demonstrated the feasibility of coulometric analysis to obtain accurate measurements of strong acid components in gas mixtures.

Purpose: To demonstrate the capability to obtain an accurate measurement of strong acid components in gas mixtures by coulometric analysis. Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry.

The state of the art technique for generating standard gas mixtures is gravimetric preparation, in which the masses of the component gases introduced into a cylinder are measured. However, for acidic gases, this approach often fails. The reason is that the introduced active gas reacts with the inside surface of the cylinder, changing its concentration in the mixture. Therefore, long-term storage of such mixtures is often impossible without change in the concentration of the acidic gas component and methods are needed to provide accurate measurements of these strong acid components in gas mixtures.

Major Accomplishments: Provided that the gas of interest can be quantitatively recovered into an aqueous solution, coulometric titrimetry can easily determine the amount of strong acid present in the solution. This approach has the advantage that long-term storage in cylinders is not required. However, it is necessary to establish that the collection procedure is quantitative. To achieve traceability, one also requires detailed knowledge of the components of uncertainty for the titration of the strong acid, as well as a traceable measurement of the amount of gas that passes through the collection system.

The analytical approach evaluated and adopted uses an automated gas manifold, two impingers, and a mass flow controller (MFC). The gas mixture to be analyzed is passed for an accurately-known collection time through the impingers via the gas manifold. Solution from the first impinger is then titrated as a dilute solution of the strong acid using the NIST automated coulometry system. To establish quantitiveness, the pH of the solution in the second impinger

is measured immediately following collection, taking care to exclude any interference from atmospheric carbon dioxide.

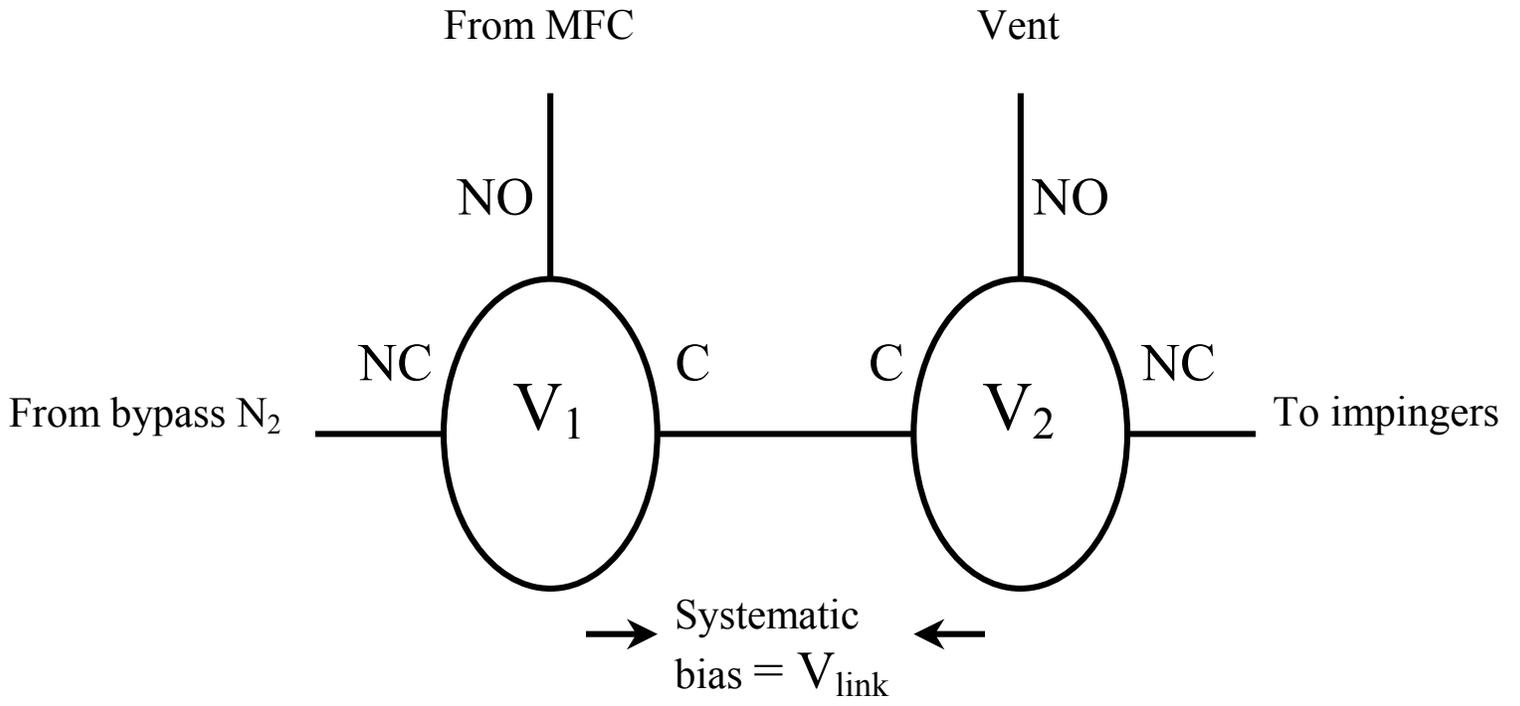
The gas manifold is shown in the attached **link**. The manifold switches between the flow of hydrogen chloride (HCl) and inert gas (nitrogen), used to purge carbon dioxide from the system and flush out the HCl lines. The manifold also supports venting of pressurized lines to the atmosphere, as required to obtain an accurate collection volume. The various states required for effecting the collection are noted in the **link**. The electronic interface that powers the solenoid valves also provides a digital timing signal used to obtain the collection time for the gas sample. The manifold is connected to the two impingers, connected in series. The concentration of hydrogen chloride in the gas mixture is obtained from the amount of acid titrated and the calibrated flow rate of the MFC. The MFC is calibrated using a traceable gas displacement system.

Initial tests demonstrated feasibility of this approach. The results of the initial tests using a hydrogen chloride in nitrogen mixture are very promising. For a 10 min collection time, a mixture with a nominal concentration of 974 $\mu\text{mol/mol}$, and a nominal flow rate of 99.1 cm^3/min (STP), the precision obtained is 0.055 % (standard deviation of the mean of 9 results). The blank (value obtained substituting nitrogen for the HCl gas mixture) is less than 0.05 % on a relative basis, using a 10 min collection time. It should be feasible to obtain similar precision levels (and overall uncertainty values) for lower concentrations with corresponding increases in the collection time.

Impact: Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry. Often, gravimetric preparation techniques typically used for other less reactive gaseous mixtures cannot be used to provide accurate composition data for acidic gas standard mixtures due to reactions such as those between the active gas and the inside surface of the gas cylinder that change its concentration in the mixture both initially and over time. Initial studies at NIST have demonstrated the feasibility of coulometric analysis to obtain accurate measurements of these strong acid components in gas mixtures.

Future Plans: Future work will be directed toward expending this principle to other acidic gases, notably sulfur dioxide and nitrogen oxides. These gases present additional difficulties, requiring close control of the chemistry of the collection procedure to ensure quantitative recovery.

Solenoid valve manifold for HCl collection



States:
 NO = 0
 NC = 1

Truth Table			
V ₁	V ₂	Output	Action
0	0	0	MFC to vent
0	1	1	MFC to impingers
1	0	0	bypass N ₂ to vent
1	1	0	bypass N ₂ to impingers

Potassium Bromate Assay by Redox Titrimetry Using Arsenic Trioxide

CSTL Programs: Chemical Characterization of Materials, Environmental Measurements

Author: *J. Smeller*

Abstract: A NIST bromate standard solution SRM is needed by environmental laboratories performing wastewater analyses and by NIST NVLAP accredited providers of proficiency testing for calibration, method validation, quality control and in pathways for demonstrating traceability to national standards. Redox titrimetry with arsenic trioxide was used to assay a high purity potassium bromate source material as the first step in the provision of this SRM.

Purpose: To assay potassium bromate, for use as a source material for a new NIST anion standard solution SRM. Many environmental labs have a regulatory need to be traceable to NIST and, increasingly, providers of commercial calibration solutions, “reference” solutions, and proficiency test materials also need a mechanism for demonstrating traceability to national standards. Bromate, one of the analytes of interest in drinking and wastewater analysis, is not currently certified as a NIST Standard Reference Material (SRM). No bromate primary standard is available. Environmental laboratories are limited to using ACS reagent grade bromate salts as a bromate source. Typically, the purity of these salts has been assayed by a titration using only two or three measurements. Only a small number of elemental impurities, usually listed as less than values, have been determined in these salts. No uncertainty information is given regarding the assay measurement, assay method, or impurities.

Major Accomplishments: An ACS reagent grade salt having a high stated purity was assayed by titrimetry and impurities covering the periodic table were determined semi-quantitatively by glow-discharge mass spectrometry (GDMS). The assay was performed by titrimetry because, as used for this analysis, it is considered a primary method. A primary method is defined as the following: having the highest metrological qualities; its operations can be completely described and understood; and, a complete uncertainty statement can be written down in terms of SI units. The method was validated for its appropriate use in this application. Potassium bromate (KBrO_3) was assayed by redox titrimetry using arsenic trioxide (As_2O_3), SRM 83d. Three aqueous KBrO_3 solutions were prepared from the KBrO_3 salt (one bottle). A nominal (0.1g) sample of As_2O_3 was dissolved in 10 mL of 5 mol/L sodium hydroxide. The solution was acidified with hydrochloric acid, and about 95 % of the KBrO_3 titrant was added gravimetrically. The end point was then determined by addition of dilute (1:3) KBrO_3 titrant using an autotitrator.

The KBrO_3 assay was determined to be $99.76 \% \pm 0.20 \%$, based on a bromate mass fraction of $76.40 \% \pm 0.15 \%$. The uncertainty considered the titrations of the three independently prepared KBrO_3 solutions. The main sources of uncertainty for each KBrO_3 solution were measurement replication, the mass of As_2O_3 , and the volume of dilute KBrO_3 .

Impact: A high purity potassium bromate source material was assayed as the first step in the provision of a new NIST SRM anion standard solution of bromate needed by environmental laboratories performing wastewater analyses and by NIST NVLAP accredited providers of proficiency testing for calibration, method validation, quality control and in pathways for demonstrating traceability to national standards.

Future Plans: Future plans include the development of the bromate anion SRM solution. The development steps include the following: preparing bromate standard solutions; evaluating the bromate solution concentrations for optimum concentration level; studying stability; and evaluating packaging.

Titrimetric assays of salts needed to develop additional anion SRM solutions may be performed using a similar analysis scheme.

Application of holistic gravimetry as a primary measurement of potassium in potassium dihydrogen phosphate

CSTL Program: Chemical Characterization of Materials

Authors: *T.W. Vetter, L.L. Yu, and J.R. Sieber; and W.F. Guthrie (898)*

Abstract: A ion-exchange gravimetric method for K in a renewal of SRM 200a, KH_2PO_4 , was developed based on a previous method for K and a similar method used for Na in serum and NaCl.¹ The ion-exchange gravimetric method used here to determine K, and previously to determine Na, was applied to minimize errors and uncertainty. The critical evaluation for this application demonstrated that the holistic gravimetry (HG) approach provided excellent accuracy (as judged by results for validation materials), an expanded relative uncertainty, coverage factor of less than 0.05 %, and a very low relative measurement replication, $u_c = 0.004$ %. A similar approach could be used to separate other analytes of interest from other matrices. Though HG is best suited for high-accuracy determinations in very pure materials, because of the relative ease of complete separation, applications can be developed for more complex matrices. A holistic approach was used to consider all sources of bias and uncertainty. This critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general.

Purpose: To continue development of gravimetric methods and critically evaluate them to determine whether they can be applied by National Metrology Institutes, NMIs, to meet the requirements of a primary method. Specifically, the gravimetric determination of potassium in potassium dihydrogen phosphate, KH_2PO_4 , by ion-exchange separation of K and its precipitation as potassium sulfate, K_2SO_4 , is evaluated and an approach called holistic gravimetry (HG) is advocated.

NMIs are interested in determining the amount of substance using primary methods. A primary method has the highest metrological qualities, its operations can be completely described and understood, and a complete uncertainty statement can be written down in terms of SI units. Gravimetric methods are commonly considered primary; however, in order to be considered primary, any gravimetric method needs to be critically evaluated to determine if analyte is lost in the procedure and if the precipitated compound is stoichiometric and free of contaminants. Many NMIs lack the knowledge and capability to apply gravimetry as a primary method. A critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general. In addition, an overall approach that is required for highly accurate gravimetry has not been specified.

Major Accomplishments: A ion-exchange gravimetric method for K in a renewal of SRM 200a, KH_2PO_4 , was developed based on a previous method for K and a similar method used for Na in serum and NaCl.¹ A holistic approach was used to consider all sources of bias and uncertainty. This approach included steps prior to the analysis such as identification of potential contaminants by semi-quantitative analysis of the KH_2PO_4 sample and designing the analysis to obtain a representative sample, reveal sample-to-sample and run-to-run differences and minimize sample loss and contamination. Each step in the determination (Figure 1) was critically evaluated and monitored for contributions to the loss of analyte or contamination of the

precipitate. Measurements were made and validation samples were analyzed to assess accuracy and determine the magnitude of any errors and the uncertainty associated with any errors. The magnitude of any undetected losses was critically estimated. Instrumental techniques, capable of determining trace amounts, were used to determine K in the fractions collected from the ion-exchange column, before and after the K fraction, and contaminants in the precipitate and in the crucibles used to ignite the K_2SO_4 precipitate.

This critical evaluation demonstrated that holistic gravimetry can be applied by NMIs as a primary method. Excellent accuracy (as judged by results for validation materials) and an expanded relative uncertainty, coverage factor (k) = 2, of 0.043 % were achieved. In addition, a very low relative measurement replication, $u_c = 0.004$ %, was obtained. Specifically, the ion-exchange gravimetric method used here to determine K, and previously to determine Na, can be applied to minimize errors and uncertainty. A separation by ion exchange, instead of the normal precipitation and filtration to separate the analyte from the matrix, leads to a relatively pure precipitate with a higher recovery of the analyte. A similar approach could be used to separate other analytes of interest from other matrices. The requirement for the ion-exchange procedure is that the analyte of interest can be easily separated from the matrix and that there be minimal retention of the analyte on the ion-exchange column after the elution of the analyte is completed. A similar gravimetric procedure as that used for Na and K, to produce a relatively pure precipitate (such as the sulfate) with a known stoichiometry, is also desirable. Though HG is best suited for high-accuracy determinations in very pure materials, because of the relative ease of complete separation, applications can be developed for more complex matrices.

Impact: A ion-exchange gravimetric method was critically evaluated and used for the determination of K in a renewal of SRM 200a, KH_2PO_4 . A similar approach could be used to separate other analytes of interest from other matrices. A holistic approach was used to consider all sources of bias and uncertainty. This critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general.

¹ J. R. Moody and T. W. Vetter, *Journal of Research of the National Institute of Standards and Technology*, 1996, 101, 155.

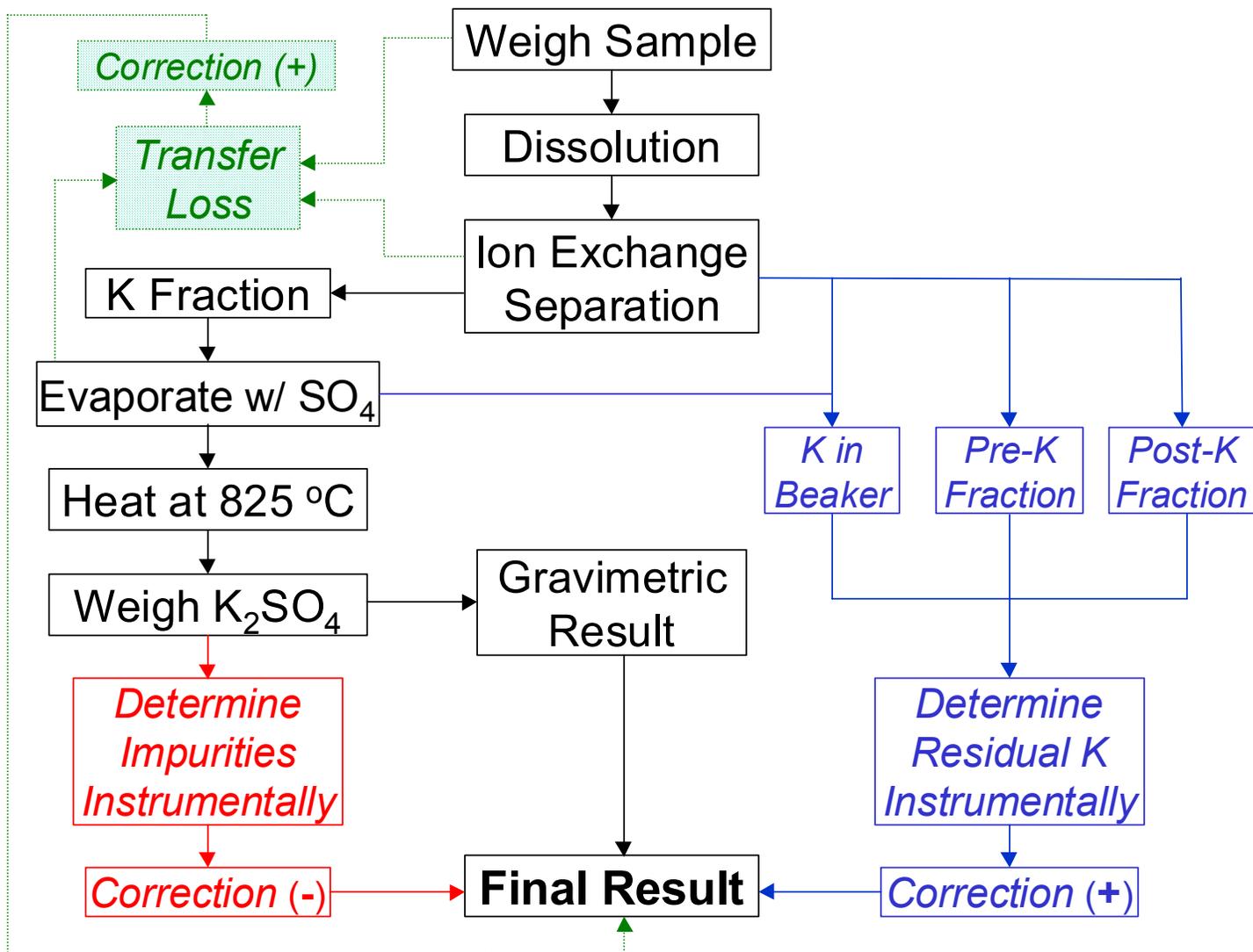


Figure 1. Schematic diagram of holistic gravimetry in the determination of w_K .

Diesel Dye Standard SRM 2037 to Assist in the Regulation and Taxation of Off-Road Diesel Fuel

CSTL Program: Chemical Characterization of Materials

Authors: P. DeRose, and G. Kramer

Abstract: Federal excise tax regulations require that tax-exempt diesel fuel, which is used in off-road vehicles, contain Solvent Red 164 (SR164) at a concentration that is spectrally equivalent to 11.1 mg/L or greater of dye standard Solvent Red 26 (SR26). Standard photometric test methods are used to determine the concentration of SR164 in diesel fuel. The accuracy and reproducibility of these methods are strongly dependent on the purity of the dye standard. Since the currently specified dye standard SR26 is not commercially available at high or consistent purity, the IRS requested NIST to secure and certify an alternative dye Standard Reference Material[®] (SRM[®]) for this purpose. NIST extensively evaluated two materials, one custom synthesized (SR 26) and one obtained commercially (SR24). Following the difficult purification process that utilized HPLC, each dye was analyzed for impurities using HPLC, ¹H NMR, ¹³C NMR, mass spectrometry, and LC-MS and then assessed for performance as a dye standard. Both appear to make equally effective standards for determining the concentration of SR164 in diesel fuel. SR24 was chosen for use as the reference material due to its ready availability and simpler purification requirements. SRM 2037 will be available in FY 2002 with certified values for purity and extinction coefficient in kerosene and xylene. Since solutions of all these Solvent Red dyes decolorize on exposure sunlight, it will be issued as a solid.

Purpose: To secure, characterize, and certify a dye standard that will enable the concentration of Solvent Red 164 dye in diesel fuel to be quantified reproducibly and accurately.

Federal excise tax regulations require that tax-exempt diesel fuel, which is used in off-road vehicles, contain Solvent Red 164 (SR164) at a concentration that is spectrally equivalent to 11.1 mg/L or greater of dye standard Solvent Red 26 (SR26) [Figure 1]. The U.S. Internal Revenue Service (IRS) enforces this regulation by (1) monitoring the concentration of SR164 in off-road fuel at refineries and fuel terminals, and (2) checking on-road diesel fuel at service stations and in vehicles for the presence of SR164. Standard photometric test methods are used to determine the concentration of SR164 in diesel fuel. The accuracy and reproducibility of these methods are strongly dependent on the purity of the dye standard. Since the currently specified dye standard SR26 is not commercially available at high or consistent purity, the IRS requested NIST to secure and certify an alternative dye Standard Reference Material[®] (SRM[®]) for this purpose.

Major Accomplishments: Two dye standard candidates, SR26 and Solvent Red 24 (SR24) [Figure 2], were acquired for study. The SR26 was custom synthesized, and the SR24 was obtained commercially. Both dyes required purification by preparative HPLC. The low solubility of SR26 in chromatographic solvents made its purification difficult. After purification, each dye was analyzed for impurities using HPLC, ¹H NMR, ¹³C NMR, mass spectrometry, and LC-MS and then assessed for performance as a dye standard. In solution, SR24 (or SR26) can be present as four equilibrating forms [Figure 3]. The overall structure consists of two tautomeric structures (azo-phenol and hydrazone), and each tautomer can have a *cis* and a *trans* isomer. The *trans* hydrazone is thought to be predominant form, but the less much prevalent *cis* hydrazone seems

to account for an early eluting HPLC peak that was initially thought to be an impurity. The precise amounts of the different forms depend on other variables such as the type of solvent, solution temperature, etc.

Highly purified SR24 and SR26 appear to make equally effective standards for determining the concentration of SR164 in diesel fuel. SR24 was chosen as the reference material (SRM 2037) due to its ready availability and simpler purification requirements. SRM 2037 will be available in FY 2002 with certified values for purity and extinction coefficient in kerosene and xylene. It is intended to replace the impure dye SR26 standards currently being used in existing test methods. Since solutions of all these Solvent Red dyes decolorize on exposure sunlight, SRM 2037 will be issued as a solid, instead of a more convenient to use set of solution standards.

Impact: SRM 2037, a Solvent Red dye, will enable the concentration of Solvent Red 164 dye in diesel fuel to be quantified reproducibly and accurately by standard photometric test methods. This facilitates the U.S. Internal Revenue Service's enforcement of regulations requiring that a Solvent Red dye be added at minimum prescribed levels to tax-exempt diesel fuel for off-road vehicles.

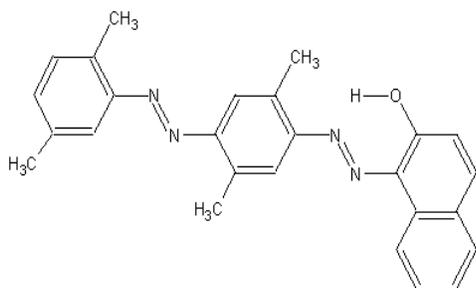


Figure 1. Solvent Red 26.

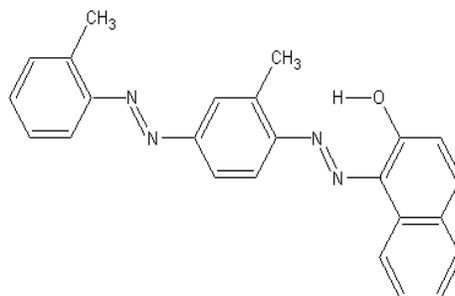


Figure 2. Solvent Red 24.

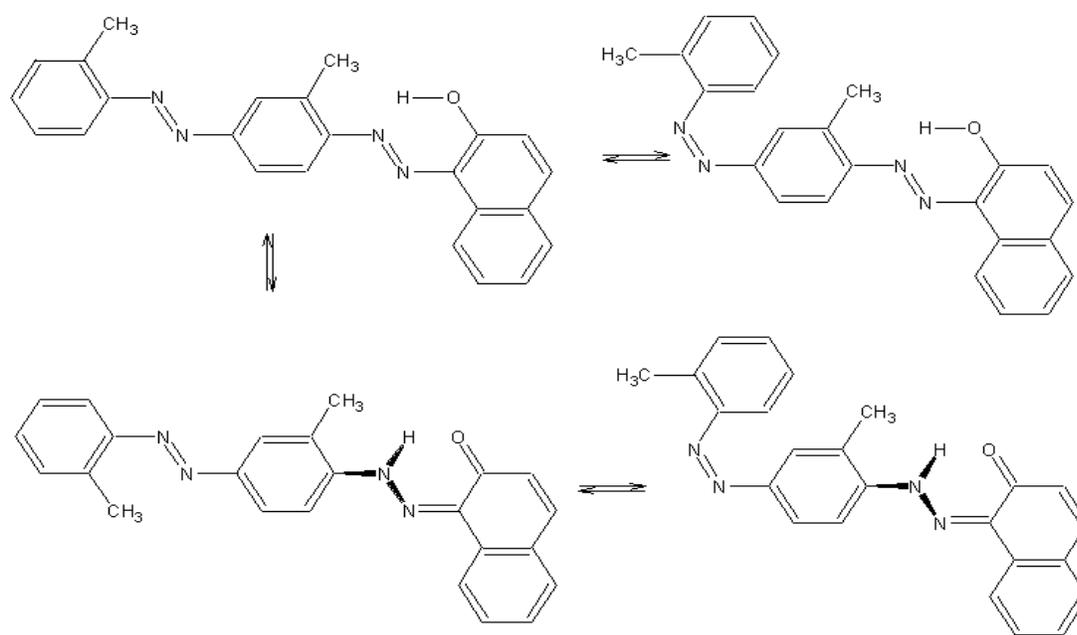


Figure 3. Equilibrating Forms of Solvent Red 24 in Solution.

Design and Applications of Plastic Microfluid Systems

CSTL Program: Nanotechnology

Authors: *L. Locascio, E. Waddell, T. Johnson, and A. Henry; D. Ross, and M. Tarlov (836); and M. Gaitan (812)*

Abstract: In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results. NIST pioneering microfabrication methods for the design and development of plastic microfluidic devices, results of its current studies on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation, and future such studies contribute to the optimization of performance, promotion, and acceptance of such devices.

Purpose: To promote the development of plastic microfluidic devices by (1) developing methods for fabrication and characterization of plastic microchannels; (2) modulating surfaces to control microflow; (3) integrating active components into microfluidic systems.

In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results.

Major Accomplishments: In recent years, our group has pioneered microfabrication methods for the design and development of plastic microfluidic devices. This year, we have focused much of our efforts on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation.

Microflow measurement is critical to the development of microfluidic devices due to the fact that the majority of measurement errors are related to flow uncertainties. As in HPLC and CE, flow fluctuations in microfluidic devices can lead to increased dispersion, decreased resolution and ultimately a reduction in device performance and system reliability. Fluctuations in temperature can also induce dispersion and flow fluctuations in microfluidic devices. We have developed several methods for characterizing flow and temperature in plastic microfluidic devices. For flow measurement, we have developed several methods including current monitoring, caged fluorescent dye imaging, and particle imaging. For temperature measurement, we have developed methods that rely on the temperature-dependent fluorescence of Rhodamine B, a common fluorophor. Also, during the past year, we have introduced techniques for controlling microflow by direct write laser micropatterning. Methods for control and modulation of microflow can be used to induce mixing or eliminate dispersion in microfluidic devices.

The development of techniques to measure bulk fluid flow rates in microfluidic channels has enabled us to provide critical data on flow properties that are materials dependent, such as EOF mobility. Measurements of dispersion have allowed us to elucidate the causes of dispersion related to different fabrication methods and different materials. Ultimately, industrial and academic researchers can use both of these measurements to predict device performance based on the substrate material and fabrication method.

New methods for temperature measurement developed in our group using fluorescence imaging and temperature dependent fluorescence of Rhodamine B allow us to measure temperature distribution in networks of microfluidic channels in real-time with high spatial resolution (Figure 1).

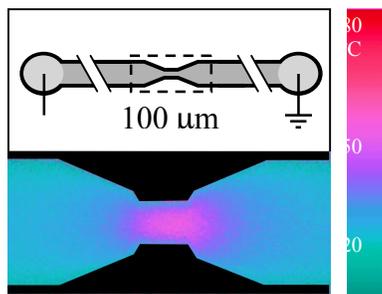


Figure 1. Temperature distribution in plastic channel.

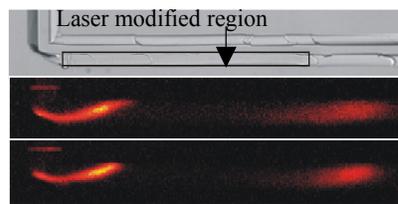


Figure 2. A. Brightfield image of microchannel with laser modified region. Fluorescence images of flow around turn in B. nonmodified channel and C.

We have also developed direct write laser micropatterning as a method to modulate flow in plastic microchannels. In this technique, portions of a preformed plastic microchannel are exposed to a UV-laser by direct write patterning thereby locally increasing the surface charge. The ability to modify the surface charge on specific surfaces within a preformed plastic microchannel allows for fine control, adjustment, and modulation of the electroosmotic flow without using wall coatings or changing the geometry of the channel to achieve the desired flow profile. We have used this approach to decrease the dispersion induced by flow around turns as shown in Figure 2.

Impact: In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results. NIST pioneering microfabrication methods for the design and development of plastic microfluidic devices, results of its current studies on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation, and future such studies contribute to the optimization of performance, promotion, and acceptance of such devices.

Future Plans: In future work, we will continue the development of micropatterning methods for application in both flow control and surface derivatization. New approaches to surface derivatization will also be used to pattern chemical selectivity into plastic microfluidic systems.

Imaging and Chemical Analysis of 100 μm Particles Through Neutron Focusing and Prompt Gamma Activation Analysis Techniques

CSTL Program: Chemical Characterization of Materials

Authors: *H.H.Chen-Mayer, G.P. Lamaze, D.F.R. Mildner, and R.Zeisler*

Abstract: Neutron radiography is a non-destructive evaluation tool for industrial materials such as aircraft components, while powerful, lacking quantitative information on elemental concentrations. Prompt gamma activation analysis can provide such information. Neutron focusing lenses can deliver a neutron beam to a spot size $< 100 \mu\text{m} \times 100 \mu\text{m}$ (FWHM), with a gain in neutron beam current density of > 40 , allowing PGAA to be performed on a small region of the sample. The smaller focal spot size enables better spatial resolution, but also makes sample alignment more challenging. We have devised a method combining neutron imaging with PGAA using monolithic capillary neutron lenses. The converging-diverging beam from the lens delivers magnified images on a neutron camera, enabling alignment of the focal spot on 100- μm samples. The measurement sensitivity for a 2.6 μg Gd sample has improved by a factor of 34. In addition to rastering samples in the lateral plane, we have also explored the possibility of profiling the inhomogeneity of the sample in the direction along the beam axis. This new tool will provide a unique capability in non-destructive analysis for industrial materials. In the future, automated scanning of lens associated with gamma ray data acquisition will give a more efficient probe for possible micro-analysis.

Purpose: Conventional neutron radiography is an indispensable tool for aircraft industry in the non-destructive evaluation of components. However, it lacks quantitative information in compositions. Prompt gamma activation analysis has been used to calibrate hydrogen in metal standards for neutron radiography, but since it is a bulk technique, the probed region must be defined to enable composition mapping. It is desirable to combine these two aspects of non-destructive analysis into a tool to investigate INTERNAL small regions of interest in a metal matrix. We have devised a method combining neutron imaging with PGAA using monolithic capillary neutron lenses for this purpose. The potential beneficiaries are the failure prevention and analysis technical areas for industry and military applications.

Major Accomplishments: Glass polycapillary fibers with hundreds of hollow micrometer-wide channels have been used for guiding and changing the direction of slow neutrons. The use of a monolithic lens for PGAA has been explored earlier. In addition, the converging nature of a focused beam can provide magnified images for neutron radiography. We combine these two uses of the focused beam from a monolithic lens and implement an alignment procedure for PGAA aided by neutron imaging.

The measurements have been performed at the end position of NG0 at the NIST Center for Neutron Research. The average wavelength of the incident beam with a Be filter is about 0.6 nm. Neutrons are imaged with a neutron camera that consists of a $\text{ZnS}(\text{Ag})/{}^6\text{LiF}$ scintillator coupled with a CCD camera with an average pixel size of 50 μm . The gamma-ray spectrometry system consists of a p-type germanium photon detector with a virtual pulser “loss free” counting module. The two monolithic lenses used in this study are both fabricated from a bundle of capillaries that is 50 mm long and with a hexagonal cross section of 10 mm wide (flat-to-flat) at the entrance and tapered to 3 mm at exit. The distance from the exit to the focus is 8.8 mm for lens A and 9.8 mm for lens B. An optimum focal spot from lens A is recorded with the imaging detector. Line profiles across the focal spot in both the horizontal and vertical directions are

taken to determine the intensity distribution and the FWHM (H: 87 μm V:100 μm) of the focus. The gain in neutron current density within the 100 μm x 100 μm area is 46 ± 2 , and within a 50 μm x 50 μm area it is 71 ± 5 .

The focused beam creates a neutron “point source”, with neutrons departing the focus within a cone of about 13° . Any neutron-absorbing object within the path of the divergent beam will cast a magnified shadow at some further distance. We make use of this effect in order to align a small sample, a Gd foil (thickness 25 μm) of size 2 mm x 0.9 mm, at the focus for PGAA (Fig. 1). We vary the distance L_1 between the sample and the lens focus by translating the lens along the direction of the beam. The magnified image enables a better alignment of the sample with respect to the focus. From the earlier measurement of the lens focus image, we determine the location of the focus with respect to the large hexagonal shadow cast by the lens entrance. We then move the lens such that the image of the sample is now superimposed on image of the focus. This guarantees that the focal point is indeed on the sample. Once the image is centered, the lens is translated to $L_1 = 0$ such that the focus is on the sample, ready for prompt gamma measurements.

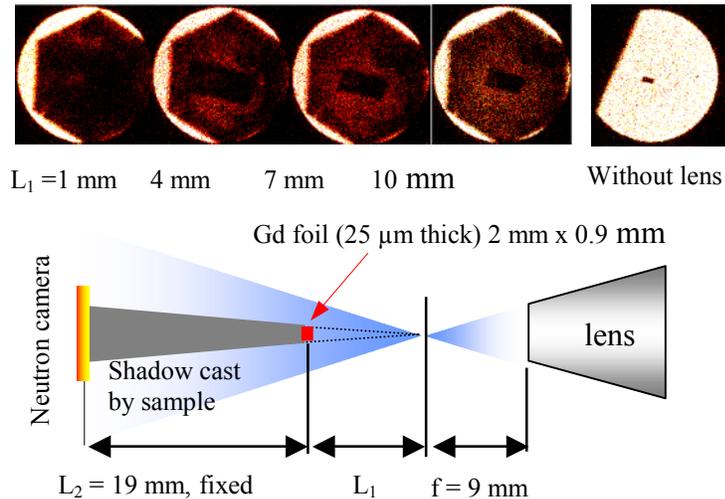


Figure 1. Magnified image of the sample in the focused beam at various distances L_1 . The hexagonal shadow is cast by the lens entrance, surrounded by the background of unguided neutrons (white). The sample is the black strip in the middle of the hexagon at various magnifications. The image of the sample without the lens is also shown for comparison.

At the center of the same Gd sample described above, there is an additional indium foil of about 1 mm x 1 mm in cross section, in contact with the Gd strip. The prompt gamma measurement without the lens gives a peak intensity for Gd at 182 keV of 2.1 counts per second (cps), but the In peak at 186 keV is not visible even for a 20-min measurement (Fig. 2, right). When the lens is used in a 10-min measurement, not only has the Gd peak intensity increased, but the In peak has the Gd peak intensity increased, but the In peak has also become visible (Fig. 2, left). Since the Gd piece is much larger than the focal spot size, the gain in sensitivity for Gd is only a factor of 4. The gain for In is not estimated.

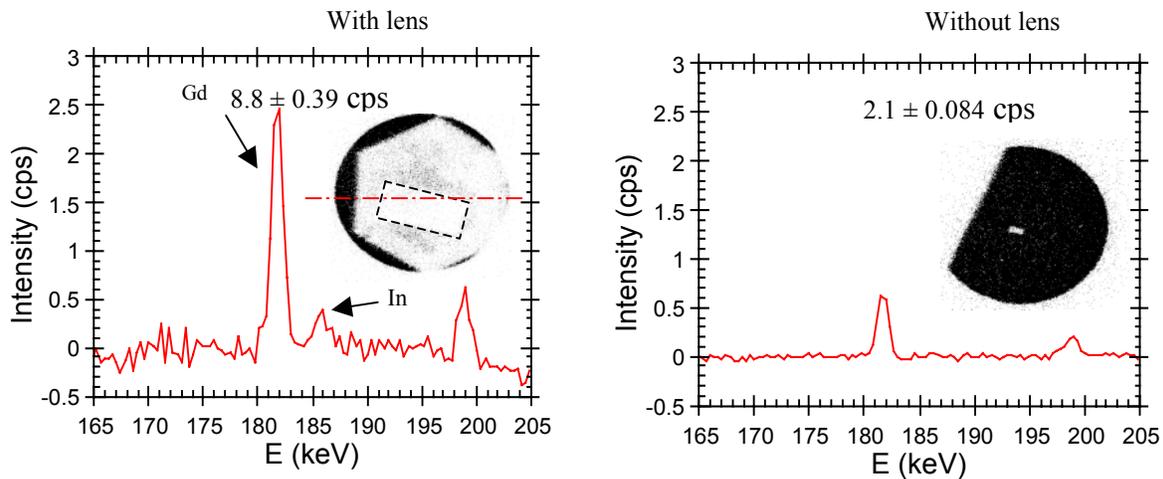


Fig. 2. Prompt gamma spectrum for Gd (182 keV) and for In (186 keV) measured with(left) and without (right) the lens, and the corresponding image of the sample. The small In signal from a small In foil attached to the Gd strip is invisible in the 20-min measurement without the lens, but becomes visible with the lens in 10 minutes.

We have also used lens B for an even smaller sample – a Gd piece of $128\ \mu\text{m} \times 103\ \mu\text{m} \times 25\ \mu\text{m}$ in size and $2.59\ \mu\text{g}$ in mass (effective mass $\sim 1\ \mu\text{g}$ taking into account self-absorption). Without the lens, the neutron camera cannot resolve the 100- μm particle. The gamma intensity with the lens is 17.4 cps with a 1.6% 1sigma counting statistics after 1 hour of counting, and with the direct beam 0.506 cps (3.9% 1 sigma) after 19.5 hours. The gain, given by the ratio of the two count rates, is 34 ± 1.4 . A scan of the lens in the vertical direction yields a peak with a FWHM of $148 (\pm 1.5)\ \mu\text{m}$, correctly corresponding to the 100- μm sample-width and the 100- μm beam-width added in quadrature.

Impact: The effort presented here is a step toward providing quantitative elemental information to traditional neutron radiography. This new tool will provide a unique capability in non-destructive analysis for civil and defense industrial materials.

Future Plans: In the future, automated scanning of lens associated with gamma ray data acquisition will give a more efficient probe for possible micro-analysis.

Bacterial Identification by MALDI-TOF MS

CSTL Program: Chemical Characterization of Materials

Authors: C. Nelson, B. Nelson, B. Porter, M. Welch, G. Mallard, and E. White

Abstract: The Biological Weapons Convention is still in the process of negotiation; however, it is clear that there is a need for credible, verifiable methods for determining if a specific organism is sufficiently pathogenic to be covered by the treaty. Development of accurate fully certified techniques for the determination of biological warfare agents will require that the variations in techniques among laboratories be reconciled. Current NIST/CSTL efforts, supported by funding from the Defense Threat Reduction Agency, are focused on standardization of methods for sample preparation and data interpretation of spectra obtain using the technique known as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), both of which are critical to further database input and search algorithm development. Essentially, proteins expressed by bacteria, both intracellularly and on the bacteria surface, can produce characteristic mass fingerprints useful in distinguishing between organisms, even at the strain level. Preliminary whole cell analysis of lyophilized bacteria indicate the presence of characteristic bacterial identification markers common among 3 strains of *Escherichia coli* tested, as well as markers that may be specific to each specific strain. Distinct mass fingerprint differences were apparent between gram negative, *Escherichia coli*, and gram positive, *Bacillus subtilis*, bacteria. Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, will aid in supporting the Biological Weapons Convention and may have wider implications for bacterial identification in other areas (*e.g.* environmental, biomedical research, clinical, food industry).

Purpose: The Biological Weapons Convention is still in the process of negotiation; however, it is clear that there will be a need for credible, verifiable methods for determining if a specific organism is sufficiently pathogenic to be covered by the treaty. The requirements imposed by the need to identify conclusively what organism is involved are expected to be quite different from the requirements for early warning in combat conditions. The development of tested and certified methods of analysis, high quality databases of organisms both pathogenic and nonpathogenic, and algorithms that will adequately distinguish analytes will be essential in the development of credible verification measures. Current NIST/CSTL efforts, supported by funding from the Defense Threat Reduction Agency, are focused on standardization of methods for sample preparation and data interpretation of spectra obtain using the technique known as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), both of which are critical to further database input and search algorithm development. Variations in the methods for analysis need to be understood, especially with respect to their roll in both positive identification and the risk of false positive identification. In this project NIST/CSTL is serving its traditional role of providing independent and objective assessment of critical measurement capabilities needed by the Nation and development of standard methods, as they are needed.

Major Accomplishments: Mass fingerprint data from lyophilized whole cell preparations of gram negative, *Escherichia coli*, and gram positive, *Bacillus subtilis*, bacteria were obtained by MALDI-TOF MS. Essentially, proteins expressed by bacteria, both intracellularly and on the

bacteria surface, can produce characteristic mass fingerprints useful in distinguishing between organisms, even at the strain level. Preliminary whole cell analysis of lyophilized bacteria indicate the presence of characteristic bacterial identification markers common among 3 strains of *Escherichia coli* tested, as well as markers that may be specific to each specific strain. Distinct mass fingerprint differences were apparent between gram negative and gram positive bacteria. Several variations in sample preparation were also investigated. Of the techniques attempted, choice of ultraviolet absorbing matrix appeared to have the largest impact on the quality of mass fingerprints produced. In addition to whole cell analysis, reproducible mass spectra, characteristic of proteins associated with the bacterial cell wall, were obtained using various solvent washes. These results are promising in identifying fingerprints that may distinguish bacteria even at the strain level.

Impact: Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, will aid in supporting the Biological Weapons Convention and may have wider implications for bacterial identification in other areas (e.g. environmental, biomedical research, clinical, food industry).

Future Plans: This work was initiated at NIST/CSTL during the past year in support of the Defense Threat Reduction Agency (DTRA), Chemical/Biological Arms Control Program (OST). Preliminary data on method protocols and acquisition of mass spectra have been obtained and the project is expected to continue for 2-3 years. NIST will continue to examine the various experimental parameters affecting the spectra of the bacteria. These will include the matrix, the intensity of the laser, the wavelength of the laser, the nature of the sample (ionic strength, growth media for bacteria, etc). Specific variability due small changes in the protocol will be examined in order to determine how exacting the specification of the standard operating procedure must be. Factors affecting reproducibility of the spectrum will be categorized as to the magnitude of the change created in the spectrum. Variation in the spectrum due to normal instrument variation will also be evaluated. A specific set of standard conditions that produce reproducible spectra under the widest set of normal instrument variations will be determined. From these experiments, standard operating procedures for the production of spectra will be developed. NIST will then create a library of MALDI-TOF MS for a wide variety of bacteria as well as bacteria that have been previously analyzed in the literature.

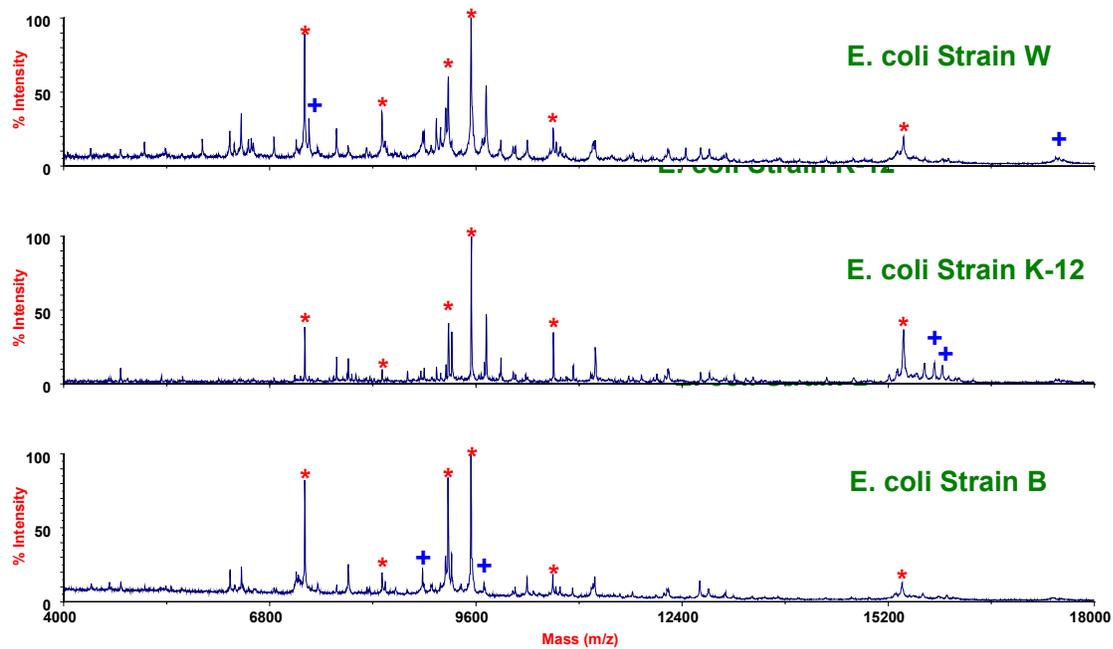


Figure 1. Whole cell MALDI-TOF mass spectral fingerprints of three strains of *Escherichia coli*. Several bacterial identification markers (BIMs) common to all three strains are indicated by “*” (red). Several BIMs that may be specific to each strain are indicated by “+” (blue).

Development of a Fluorescein Solution Fluorescence Standard to Facilitate Quantitation in Clinical Diagnostics and Biotechnology

CSTL Programs: Healthcare Measurements, Chemical and Biochemical Sensing

Authors: *P. DeRose, A. Gaigalas, L. Wang, and G. Kramer*

Abstract: Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. SRM 1932, fluorescein standard, being developed and to be issued in FY2002, is a fluorescent concentration standard that will provide a viable alternative to an absolute fluorescence intensity standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use. In addition, a fluorescence standard composed of a fluorescein-labeled microbead suspension is presently being developed. These microbeads mimic biological cells labeled with fluorescein. Therefore, this microbead solution standard will serve as a fluorescence intensity calibration standard in biological assays using fluorescein.

Purpose: To prepare, characterize and certify a fluorescent standard composed of a fluorescein dye solution to facilitate the calibration of fluorescence instrumentation.

Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Since a measured fluorescence intensity is dependent on the detection system of the instrument used, as well as the incident light intensity, it is difficult to put fluorescence intensity on an absolute scale. This greatly discourages the comparison of fluorescence intensities between instruments. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. A viable alternative to an absolute fluorescence intensity standard is a fluorescent concentration standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. These concentration standards will enable fluorescence instruments, such as flow cytometers, fluorometers, fluorescence chip readers, etc., to be calibrated for fluorescence intensity for a particular fluorophore. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use.

Major Accomplishments: A commercial source of high purity fluorescein powder has been secured. Its purity has been determined using several analytical techniques, including HPLC, MS, NMR, elemental analysis, etc. We have prepared a fluorescein solution in borate buffer at pH 9.1. Its concentration has been determined gravimetrically and its extinction coefficient has also been measured.

SRM 1932 fluorescein standard will be available in FY2002 with a certified value for concentration. Fluorescein solution decomposes when exposed to light, heat or oxygen, so SRM

SRM 1932 will be composed of a set of five, single-use, amber glass ampoules that are flame sealed under argon. They should be stored in the dark at about 5 °C. The stability of SRM 1932 over time has been tested at regular intervals for more than a year and will continue to be tested for at least another year. No measurable changes in fluorescence intensity or absorbance have been observed to this point. A fluorescence standard composed of a fluorescein-labeled microbead suspension is presently being developed. These microbeads mimic biological cells labeled with fluorescein. Therefore, this microbead solution standard will serve as a fluorescence intensity calibration standard in biological assays using fluorescein.

Impact: Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. SRM 1932, fluorescein standard, to be issued in FY2002, is a fluorescent concentration standard that will provide viable alternative to an absolute fluorescence intensity standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use. A fluorescence standard composed of a fluorescein-labeled microbead suspension, also being developed will serve as a fluorescence intensity calibration standard in biological assays using fluorescein as the microbeads mimic biological cells labeled with fluorescein.

Future Plans: The stability of SRM 1932 over time has been tested at regular intervals for more than a year and will continue to be tested for at least another year.

New Thermal Neutron Prompt Gamma Ray Activation Analysis Instrument

CSTL Program: Chemical Characterization of Materials

Authors: E.A. Mackey, and R.M. Lindstrom; D.L. Anderson (US FDA); and P.J. Liposky (856)



Abstract: A new thermal neutron prompt gamma ray activation analysis instrument became operational in FY01. The new Ge detector has a resolution of 2.0 keV (for the 1332.5 keV line from ^{60}Co) and 40% relative efficiency (relative to a 7.6-cm cube of NaI). A sapphire filter was placed in the neutron beam shutter assembly and all external components of the original system were replaced. The new beam tube is made of two sections, constructed from aluminum lined with a lithiated polymer (lithoflex). Both sections are sealed at each end, and kept under vacuum. The sample chamber is also constructed of aluminum and lined with lithoflex. The chamber may be evacuated when necessary to eliminate neutron scattering by air. Experiments were performed to measure mass fractions of Cd in SRM 2702 Inorganics in Marine Sediment, and of B, K and Cl in SRM 1575a Pine Needles. Additional experiments were

performed to determine the background count rates during various operational conditions, to measure element sensitivities in this configuration, and to determine the optimum amount of neutron and gamma ray shielding that will be incorporated into the final design for the detection system. Future work will involve optimization of the detection system and shielding configuration, design and fabrication of a variable position detector support platform, and complete characterization the new system.

Purpose: This instrument has been upgraded to improve analytical sensitivities and limits of detection, and decrease the levels of radiation associated with use of the instrument. This was achieved by minimizing gamma rays created by neutron capture in the components of the instrument and support structure, and by installing an improved detection system.

Major Accomplishments: A cylindrical 5.3-cm long (4.3-cm diameter) sapphire beam filter was incorporated into the shutter assembly. This modification reduced the number of fast neutrons by a factor of five, decreased the thermal neutron flux by approximately 12%, and greatly reduced low-energy gamma-ray background. The design and fabrication of the new beam tube, beam stop, sample chamber and support structure was completed. The sample chamber and beam tube were fabricated from aluminum, and lined with lithoflex. The beam tube is kept under vacuum to reduce background gamma-rays arising from neutrons scattered by air and captured in the surrounding materials. The beam stop consists of an aluminum box welded onto the aluminum

support structure, and is filled with a cube of borated polyethylene surrounded by lead. A new Ge detector with improved resolution and peak-to-Compton ratio was installed. This detector has a relative efficiency of 40% (relative to a 7.6 cm cube of NaI) and 2.0 keV (peak full-width-half-maximum) resolution for the 1332.5 keV gamma ray line from ^{60}Co . Element sensitivities obtained using this new detection system with the new instrument are approximately 1.7 times greater than those obtained with the previous instrument and detection system. Background count-rates for H decreased by approximately a factor of five, and those for N are reduced by a factor of five when the sample chamber is evacuated. Replacing the NaI Compton suppression system with a bismuth germanate suppressor eliminated Na gamma-ray background but increased the background from neutron capture in Ge. In the future, the Ge background count-rate will be reduced by increasing the amount of neutron shielding surrounding the detector.

Impact: The design changes have greatly improved the signal-to-noise ratio in the low energy region. With this improved system, we will be able to determine mass fractions of several rare earth elements that could not be measured using the previous system, which was limited because of much higher background in the low energy region. Detection limits for H have been significantly improved because the background count-rate for this element has been lowered by a factor of 10. Improvements to the detection system, overall, have provided a twofold improvement in element sensitivities. This new instrument will allow much better characterization of biological, environmental, fuel, and various other materials. During FY01, the instrument was used to determine the mass fraction of Cd in SRM 2702 Inorganics in Marine Sediment, and mass fractions of B, K, and Cl in SRM 1575a Pine Needles.

Future Plans: In FY02, the design and construction of the detector shielding will be completed. A bismuth germanate Compton suppressor will be added to the detection system to reduce background gamma radiation associated with Compton events. The design and fabrication of a variable position support structure for the detection system will be completed.

Accurate Measurement of the Half-life of ^{76}As

CSTL Program: Chemical and Biochemical Data:

Authors: *R.M. Lindstrom; M. Blaauw (Delft University of Technology, The Netherlands; and R.F. Fleming (The University of Michigan, Ann Arbor)*

Abstract: In the course of high-accuracy measurements of arsenic, we found that the most recently published and compiled half-life of ^{76}As did not fit our data as well as the earlier accepted value. To redetermine this parameter, five ^{76}As sources were measured on four Ge detector systems, and the decay data fit to an exponential function by two different nonlinear least-squares methods. We determined $t_{1/2} = 1.0938$ d with an expanded uncertainty $U = 0.0009$ d. This result is 1.5% higher than the recent value, and in agreement with the older, less precise, consensus value.

Major Accomplishments: Knowledge of half-lives for radioactive decay is necessary for all applications of radioactivity. In a few cases (such as the neutron and Ti-44) accurate knowledge is needed in grand problems like the Standard Model and supernova kinetics, but in most cases moderate accuracy suffices. However, in the course of high-accuracy measurements of arsenic in silicon we found that the most recently published and compiled half-life of As-76 did not fit our data as well as the earlier accepted value. The discrepancy between old and new recommended values amounted to 5% at the end of the 4-day experiment, an unacceptable error when we were seeking an overall (1s) error of 0.3% or better. We therefore used the old 1972 value reported by Emery at ORNL, because it is the mean of seven measurements with six different kinds of detectors and because it fit our data well. The 1994 measurement was made by Ge spectrometry and published with more experimental detail than Emery had done.

Evaluation and publication of nuclear data in the USA is centered chiefly at Lawrence Berkeley and Brookhaven National Laboratories. The primary data from the literature are collected in a database called the Evaluated Nuclear Structure Data File (ENSDF). Periodically the data for a given mass number are evaluated, and recommended values are published in Nuclear Data Sheets and in the Table of Isotopes. The 1994 value was adopted because the uncertainty analysis in that paper was more complete than in older publications.

During the past year we have returned to this issue. Using several detectors and two styles of nonlinear least-squares analysis of the data, we have re-measured the half-life, with precision (and inferred accuracy) an order of magnitude better than Emery's measurement. Our result and his overlap within the 1s uncertainties, but we both disagree completely with the 1994 value.

To verify our methods, we also measured the half-life of Au-198, one of the best-studied nuclides because of its fundamental importance in the unification of the energy and wavelength in photon spectroscopy. Our number agrees with the most precise (and adopted) value to within 0.1%. The problem with the 1994 paper is that the author fitted the $\log A$ vs t rather than A vs $\exp(-\lambda t)$; the former is to lead to half-lives that are too short, just what the 1994 paper shows for the four nuclides reported (Na-24, K-42, As-76, and Au-198).

A brief account of our work is now in NIST review for publication.

Impact: An erroneous published value for a physical parameter has been corrected. Correspondence with scientists involved in data evaluation has led to a revision of the accepted value of the As-76 half-life, and has led to the establishment of a new algorithm for combining data that is superior to the one previously in use.

Future Plans: A detailed publication on the NIST-IRI methodology is in preparation. Now that the weaknesses of the published data have been revealed and the power of the NIST methodology have been established, a continuing low-level effort to redetermine a selected set of half-lives will be carried out, in collaboration with the Ionizing Radiation Division.

Mechanisms for the Delivery of Traceability and Quality Assurance for Chemical Measurements

CSTL Programs: Chemical Characterization of Materials, Healthcare Measurements, Environmental Measurements, International Measurement Standards

Authors: *Analytical Chemistry Division Staff*

Abstract: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). These mechanisms include:

- Quality Assurance and Traceability with SRMs
- Quality Assurance and Traceability through Definitive/Reference Methods
- Quality Assurance and Traceability via Standard Reference Data
- Quality Assurance and Traceability through NIST Traceable Reference Materials
- Quality Assurance and Traceability using Standard Reference Instruments
- Quality Assurance via Proficiency Testing

We continue to search for new mechanisms to most appropriately meet strategic, high priority quality assurance and traceability needs of our customers.

Purpose: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). While many are familiar with our most visible mechanism for these tasks—Standard Reference Materials[®] (SRM[®]s)—, ACD has long provided other, less well known approaches, has recently developed new methods, and continues to evolve new schemes to benchmark chemical determinations by leveraging NIST measurement expertise.

Major Accomplishments:

Quality Assurance and Traceability with SRMs

SRMs have traditionally been the primary tools provided by NIST for achieving chemical measurement quality assurance and traceability to national standards. Of the nearly 1300 different types of SRMs offered for sale, about 700 are certified for chemical composition. In 2001, such “chemical” SRMs accounted for ~16,000 of the 32,000 SRM units sold, and the large majority of these SRMs were certified by ACD staff. Most SRMs are certified in batches where value assignments and measurement uncertainties are determined from measurements on a statistically significant number of samples. In a few instances (e.g., gas mixtures and optical filters), SRMs are individually certified. In some of these cases, ACD provides recertification services to extend the useful life of these standards. In FY '01, 176 optical filter SRMs were recertified for 125 different customers and 104 gas cylinder SRMs were recertified for 13 companies.

Quality Assurance and Traceability through Definitive/Reference Methods

The National Committee for Laboratory Standards (NCCLS) established the National Reference System for Clinical Laboratories (NRSCL) to provide a traceability structure for clinical laboratory measurements. Part of this system was establishment of a hierarchy of methods with three levels: definitive, reference, and field. Field methods are the routine methods used in clinical laboratories. Reference methods are more complex methods that are generally within the capabilities of many laboratories and are used to directly provide traceability to the field methods. At the top of the hierarchy are the definitive methods. These methods use techniques that are considered to be primary methods by international metrological organizations and provide accurate and precise results with complete uncertainty statements. NIST's role in the NRSCL is to develop and maintain definitive methods for clinical analytes and to apply these methods to the certification of SRMs and other certified reference materials (CRMs), which are then used to test the accuracy of reference and field methods. No other U.S. laboratory develops and maintains definitive methods and no other national metrology institute has as extensive a program as does NIST for such high level methods.

In the development of the NRSCL, NIST took a leadership role in developing and evaluation of the reference methods (initially called referee methods) as well as the definitive methods applied at NIST. The first example of this was for the element calcium and the theory and practice for the successful development of a reference method was expounded in this work. The candidate reference method was an atomic absorption spectrometric method that had been published in Clinical Chemistry. The method was developed by a team of experts from clinical chemistry and evaluated with eight clinical chemistry laboratories. The conclusion of the effort was that "subject to the limitations and provisos stated, calcium in serum can be determined by the referee method to within ± 2 % of the 'true or absolute' value." Reference methods were initially evaluated in conjunction with the establishment of many of the definitive methods. The results have been published in the NBS Special Publication 260 series. [NBS SP 260-36, "A Referee Method for the Determination of Calcium in Serum"; NBS SP 260-60, "A Reference Method for the Determination of Sodium in Serum"; NBS SP 260-63, "A Reference Method for the Determination of Potassium in Serum"].

For some applications, definitive methods are either not possible or not practical. In such cases, NIST focuses on development of reference methods that are critically evaluated. When it is feasible, NIST will develop two independent reference methods for a given analyte and use results from both methods for certification of SRMs. If it is not possible or practical to develop a second independent method, NIST generally organizes an interlaboratory study with laboratories that have considerable experience with measuring the analyte in a matrix similar to that of the SRM. Whenever possible, NIST incorporates existing SRMs or other CRMs into the measurement schemes to serve as controls.

The ultimate goal of any chemical measurement traceability scheme is to show a linkage to the mole. There are substances where such a scheme is not possible, e.g., proteins and other complex biomolecules with variations in structure, but it is possible for most substances for which chemical measurements are performed. The requirement is that there must be a well-characterized substance, either elemental or a compound, with a known purity, that can be

weighed and used as a primary reference material to calibrate reference and definitive methods. NIST uses an arsenal of techniques to establish the purity of the substances used as primary references, many of which are SRMs. Uncertainties in the results from the best analytical techniques can be no better than the uncertainty in the purity of the primary reference material used.

Quality Assurance and Traceability via Standard Reference Data

Many analytical techniques, especially spectroscopic methods, work by measuring a parameter that can be related to chemical concentration through the use of a material dependent constant. For example, quantitative spectrophotometry relies on the Beer-Lambert-Bouguer law that states that the optical absorbance of a solution of a given material is proportional to the pathlength of observation and the concentration of the material. The material property that relates concentration to absorbance at constant path length is called the absorption coefficient. Given the ability to make high quality determinations of the physical parameters (in this case absorbance and pathlength), chemical concentration measurements then depend on having highly accurate determinations of the material property (here the absorption coefficient). NIST has long provided such data through its Standard Reference Data (SRD) program. A SRD Quantitative Infrared Database (SRM 79) was developed in ACD to provide accurate absorption coefficients for the measurement of gaseous chemical emissions and hazardous air-pollutants(HAPs) (see separate TAR). SRD 79 provides absorption coefficient data for 40 HAPs on the U.S. EPA priority list. These data were critically evaluated from measurements made on gravimetrically produced gas samples and provide a solid foundation for high quality open-path field monitoring methods.

Quality Assurance and Traceability through NIST Traceable Reference Materials

Although NIST produces SRMs, it is not a production facility. There are instances where the demand for certain SRMs overwhelms our abilities to produce them. In some cases, the use of an SRM is mandated by statute or by the sheer magnitude of the financial consequences of certain chemical measurements. Environmental gas standards were propelled to that status by the passage of the Clean Air Act that permitted emissions trading. In response to this sudden demand for traceability in gas measurements, ACD created the first NIST Traceable Reference Materials (NTRM) program. Although the details of operation differ among NTRM programs, the basic notion is that NTRMs are produced and certified by commercial vendors with NIST providing an overseeing quality assurance function. The NTRM mechanism is appropriate where there is sufficient economic incentive to justify it and sufficient measurement control to make it feasible.

The Gas Mixture NTRM Program was initiated to be real time response to the needs of the Specialty Gas Industry (SGI). The Specialty Gas Companies (SGC) constantly monitor the needs of the end user community and determine what mixtures are needed to fill the needs. The required mixtures are then prepared in batches of ten or more cylinders by the SGC, analyzed, and data submitted to NIST. NIST selects 10% of the cylinders in the batch and analyzes them so that NIST can value assign the batch. The cylinders are returned to the SGC with Certification documentation for the batch. The cylinders are under control of the SGC and can be sold to end users to provide a NIST traceable gas or the NTRM mixtures can be used by the SGC to analyze other mixtures that are then sold to provide traceability. The qualifier on the part

of NIST is that NIST must have the primary standards available to perform the NIST analyses. In FY '01, 36 batches of NTRMs were certified for four different companies.

The NIST Optical Filters NTRM program provides absorbance standards for visible spectrophotometry. Currently, this program is just getting underway with three vendors. In the Optical Filters NTRM program, each vendor is certified by the National Voluntary Laboratory Accreditation Program (NVLAP) to produce visible filter standards. Traceability is provided by the use of NIST SRM optical filters to calibrate vendor spectrophotometers, and quality assurance is afforded by NIST review of all vendor calibration, certification, and recertification data, NIST measurements (one set from each vendor production batch), and blind spot checks.

Quality Assurance and Traceability using Standard Reference Instruments

Providing a reference materials for very reactive or unstable chemical entities is particularly challenging. In certain cases, traceability and quality assurance can be provided through standardization of the instrumentation used to make the measurement. Since 1983 NIST has provided Standard Reference Photometers (SRP) for determining the concentration of ozone in air (see separate TAR). Based on UV photometry, ten ozone SRPs form the basis of the U.S. EPA's infrastructure to monitor ozone. Thirteen additional SRPs are now located around the world. Each of these SRPs was built and calibrated at NIST and is returned periodically to NIST for recalibration against one of two master SRPs maintained at NIST. In addition to providing SRPs, NIST offers a service that calibrates commercial ozone photometers against one of the NIST master SRPs. As the need for ozone measurements becomes more international, it seems appropriate that the primary reference standard be international rather than national. Accordingly, NIST is now working with the BIPM to develop a primary reference standard for ozone that can anchor all ozone measurements through a defined traceability structure.

Quality Assurance via Proficiency Testing

Ensuring that the providers of chemical measurements services actually provide accurate results is an important quality assurance issue everywhere, but it is a critical issue in regulated industries, such as clinical and environmental laboratories. In general NIST does not consider the testing of service laboratories to be within its purview. However, it is a NIST role to ensure that such testing is accurately done. Accordingly, NIST becomes involved in "testing the testers." ACD is beginning to provide this service for environmental laboratory Performance Test (PT) providers. PT providers offer performance evaluation services to individual laboratories by sending samples to the laboratory for analysis and evaluating the results. NIST helps provide a level playing field by providing NVLAP accreditation for PT providers and by assuring the quality of the PT samples. Traceability is provided through the use of NIST SRMs similar to the PT samples, while quality assurance is provided by ACD measurements on samples from the PT batches sent to NIST by the PT providers.

NIST has helped improve the state of the practice in certain areas by using a combination of its quality assurance and traceability tools. One example of this is the on-going Micronutrients Measurement Quality Assurance Program. In this program, ACD conducts interlaboratory test studies on ACD-analyzed samples sent from NIST to participating laboratories. Recent studies included one for vitamin C and two on fat-soluble vitamins. After analyzing the samples, the participating laboratories send their results to NIST where they are statistically evaluated.

Results are made available individually to each participant, and the collective results are presented at a group meeting. For laboratories with results outside the acceptable range, NIST provides consultation and assistance on analytical techniques. Using this mechanism, the spread of analytical values among the participants has narrowed markedly over the years.

A New Traceability Tool

The accuracy of measurements of lead concentration in blood, or arsenic in soil, or sulfur in an aircraft alloy, are all dependent on the availability of accurate elemental solution standards for calibration. Such standards are available from NIST as the SRM 3100 Series, but the volume of demand for such standards worldwide is too great for NIST to play a significant role as a direct supplier of such standards. Most laboratories purchase their standards from a commercial source, but require that these standards be traceable to the NIST SRM 3100 Series. It is here that these SRMs play their most important role as national standards for element concentration.

The certificates that accompany commercial elemental solution standards generally include a statement on the traceability of the standard to the matching SRM from the 3100 Series. A valid claim of such traceability requires a comparison measurement between the traceable standard and the SRM, with a stated measurement uncertainty. The uncertainty of the value of a standard that is traceable to an SRM must include the uncertainty of the SRM (typically 0.3 %) together with the uncertainty of the comparison measurement. Ideally this measurement uncertainty would be much less than the SRM uncertainty, but this is beyond the current capability of most laboratories using instrumental measurements. We have developed the technique we call High-Performance ICP-OES with the capability of performing comparisons between solution standards with relative standard uncertainties of 0.03 % using unmodified commercially available ICP-OES instrumentation, and are promoting its use by standards providers who wish to establish traceability between their standards and the SRM 3100 Series. As an aid to implementing this comparison method we have created an interactive spreadsheet-based “traceability tool” to guide an analyst through the procedure. This tool has been tested by several standards providers, who have seen significant reductions in the uncertainty of their ICP-OES comparison uncertainties using their existing instrumentation. Traceable analyte mass fractions have been assigned to standards with uncertainties that are virtually identical to the uncertainties of the SRM.

Impact: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). These efforts impact U.S. industry's productivity and competitiveness as well as provide the measurement and standards infrastructural support for environmental quality and human health assessments.

Future Plans: In ACD, we continue to search for new mechanisms to provide quality assurance and traceability to our customers. For example, an international intercomparison exercise conducted this year has given rise to a notion of “intrinsic “ standards that combine concepts from both SRD standards and reference methods. This idea is still evolving and will require further definition and research to determine if and/or where it is practical. Given the breadth of

the U.S. economy and the diversity of our customer base, NIST will always have too much to do. Only by constantly re-evaluating our methods, leveraging our measurement capabilities, and continuing to develop new ways of delivering our services can we hope to keep up and push both the state of the art and the state of the practice in chemical measurements.

A NORAMET Evaluation of Holmium Oxide Solution Reference Materials for Wavelength Calibration in Molecular Absorption Spectrometry

CSTL Programs: Healthcare Measurements, Chemical Characterization of Materials, International Standards

Authors: *J.C. Travis, D.L. Duewer, M.V. Smith, M.D. Maley, and G.W. Kramer*

Abstract: Molecular absorption spectrometers operating in the ultraviolet and visible spectral regions (“UV/vis” spectrophotometers) are widely used in clinical laboratories and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. NIST supplies a suite of SRM[®]s designed to help users of UV/vis spectrophotometers ensure the validity of their measurements. Recently, NIST conducted a study among five laboratories within the three NORAMET NMIs to assess the current degree of comparability of wavelength measurements among these participants under “routine” operating conditions using commercial instrumentation and between the aqueous Ho₂O₃ wavelength standard produced by NIST (SRM 2034) in the U.S. and that provided by CENAM (DMR 41) in Mexico. The study also produced Ho₂O₃ solution spectral data from a variety of spectrometers for use in a NIST study of wavelength assignment algorithms and to provide a basis for a possible reassessment of the NIST-certified wavelength values. The resulting data show a substantial level of comparability across laboratories, measurement events, CRM preparations, and peak location algorithms. The results demonstrated that the wavelength comparability of the five participating instruments actually could be improved by calibrating all of the instruments to the consensus band locations of Ho₂O₃ solution. This finding supports the value of absorption-based wavelength standards for absorption spectrophotometers.

Purpose: To demonstrate comparability of wavelength measurements among the three National Metrology Institutes (NMIs) of the North American Cooperation in Metrology (NORAMET) and between the aqueous Ho₂O₃ wavelength standard produced by NIST (SRM 2034) in the U.S. and that provided by CENAM (DMR 41) in Mexico. The comparability study also was designed to acquire Ho₂O₃ solution spectral data from a variety of spectrometers for use in a NIST study of wavelength assignment algorithms and to provide a basis for a possible reassessment of the NIST-certified wavelength values for SRM 2034.

Molecular absorption spectrometers operating in the ultraviolet and visible spectral regions (“UV/vis” spectrophotometers) are widely used in clinical laboratories and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. The NIST Chemical Science and Technology Laboratory supplies a suite of SRM[®]s designed to help users of UV/vis spectrophotometers ensure the validity of their measurements.

Holmium oxide (Ho₂O₃) in dilute acidic aqueous solution provides a safe, convenient, and effective signal for UV/vis wavelength calibration, having numerous fairly narrow and symmetric absorption bands well distributed over the wavelengths of interest [Figure 1]. NIST SRM 2034, Holmium Oxide Solution Wavelength Standard from 240 nm to 640 nm, was first produced and certified in the mid-1980s. Since spectrophotometric instrumentation and practice

have become vastly more “computerized” since that time, NIST conducted this NORAMET study to reevaluate the performance of aqueous H_2O_3 Certified Reference Materials (CRMs) under “routine” operating conditions using commercial instrumentation. This study was not intended to intercompare national wavelength scales but to demonstrate comparability of wavelength measurements among the participants and between the NIST and CENAM CRMs.

Major Accomplishments: In the NORAMET study, aqueous H_2O_3 CRMs were measured by five laboratories within the three National Metrology Institutes (NMIs) of the North American Cooperation in Metrology (NORAMET) under “routine” operating conditions using commercial instrumentation. The resulting data show a substantial level of comparability across laboratories, measurement events, CRM preparations, and peak location algorithms. At the same time, it demonstrated that the wavelength comparability of the five participating instruments can actually be improved by calibrating all of the instruments to the consensus band locations of H_2O_3 solution [Figure 2]. This finding supports the value of absorption-based wavelength standards for absorption spectrophotometers. Coupled with the demonstrated robustness of the band position values with respect to preparation and measurement aberrations, it also supports the concept of extending the present approach to additional NMIs in order to certify a properly prepared H_2O_3 solution as an intrinsic wavelength standard; i.e., utilizing centralized data analysis at NIST of well-calibrated raw spectra accumulated by well qualified personnel from selected NMIs. The use of a variety of high-end spectrophotometers – in qualified hands – has permitted a much more realistic evaluation of uncertainties than is possible from any single instrument.

Impact: “UV/vis” spectrophotometers are widely used in clinical medicine and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. The NORAMET evaluation demonstrated a substantial level of measurement comparability among NORAMET NMIs and of the NIST and CENAM certified reference materials used to calibrate these instruments.

SRM 2034: <http://srmcatalog.nist.gov/nist/products.nsf/1117b1be0285f3bc85256a180051cc88/412c4d6e3a20c46e85256a2b0045e477?OpenDocument&Highlight=0,2034>

NORAMET: http://www.nrc.ca/inms/int_coop/noramet.html

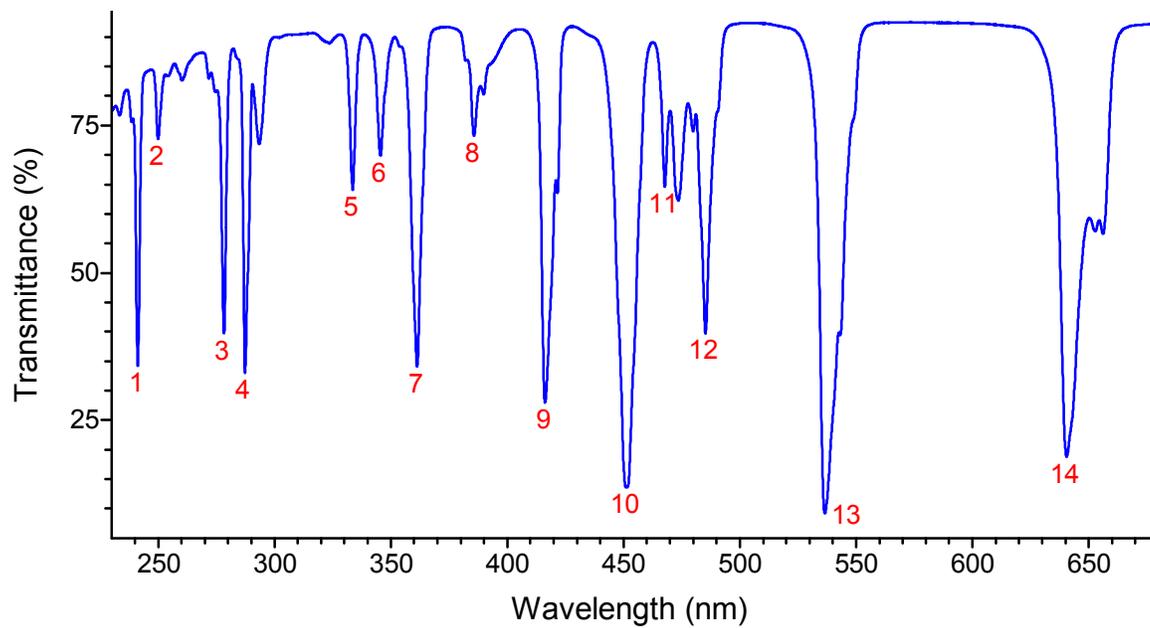


Figure 1. Spectral transmittance at 1 nm spectral bandpass of a 4 % solution of holmium oxide in 10 % perchloric acid solution. Bands having certified SRM® 2034 and DMR 41 locations are labeled “1” through “14”.

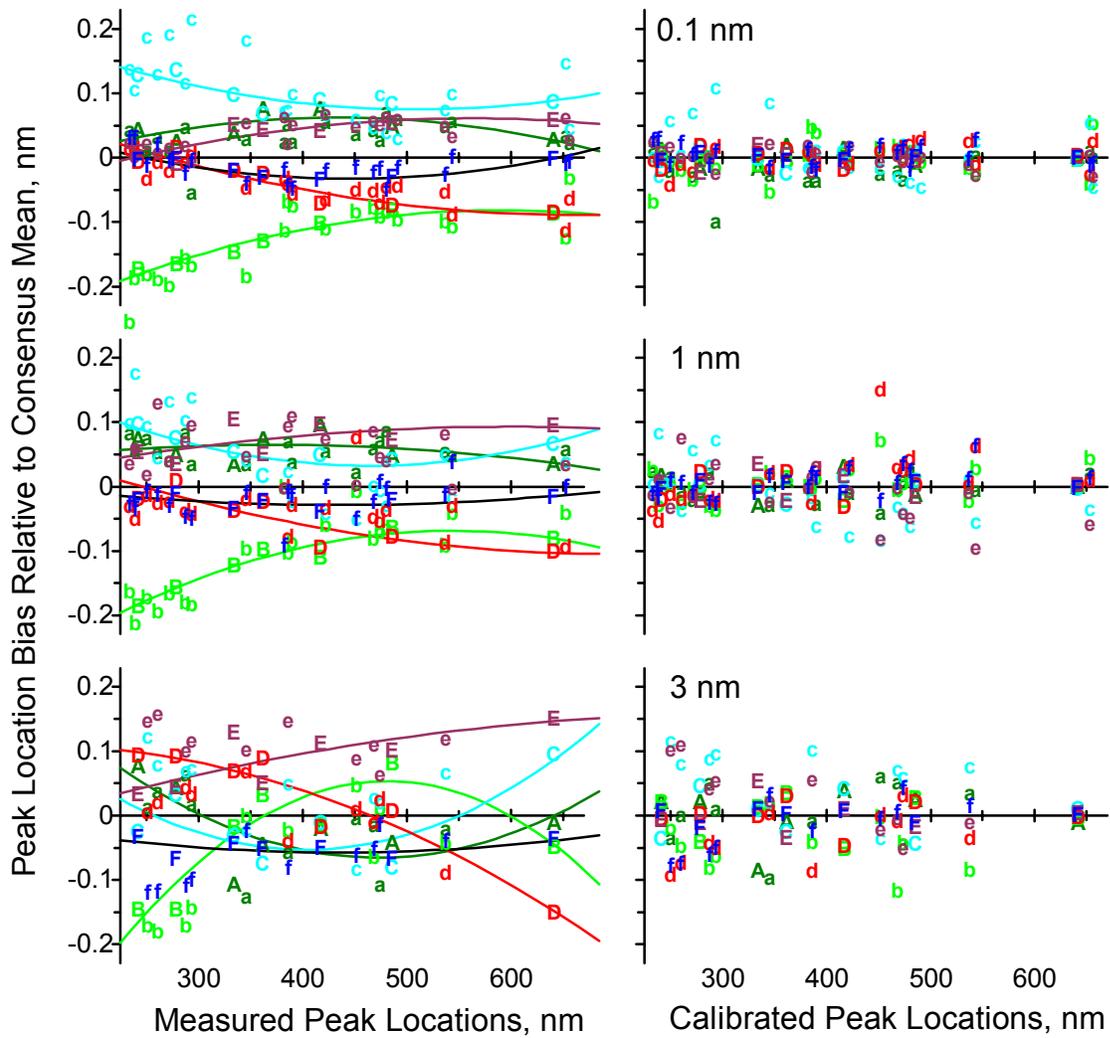


Figure 2. The three left-side segments display the among-set biases of the measured locations relative to the consensus mean for the 0.1 nm, 1 nm, and 3 nm spectral slit width (SSW) spectra. Biases are estimated as the location difference averaged over sets of eight spectra at each SSW. Quadratic polynomial calibration curves of the measured biases to the consensus locations are also displayed. The capital symbols denote the seven features used to define the calibrations. The three segments to the right display the bias data after adjustment to their calibration curve.

SpectroML—an Extensible Markup Language for Molecular Spectroscopy Data

CSTL Program: Chemical and Biochemical Data

Authors: *M.A. Rühl, M.G. Peschke, J.C. Travis, R. Schäfer, and G.W. Kramer*

Abstract: Today's analytical chemists need not only to interchange data generated within a specific technique, they, their organizations, and their colleagues need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. In the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange. Over the past year, we have developed a “web-aware” mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectrometry data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectrometry and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers among ourselves, our colleagues at other national metrology institutes, and our NIST-Traceable Reference Material (NTRM) vendors. We created SpectroML in the Extensible Markup Language (XML) and defined and documented it with both a DTD (Document Type Definition) and a schema. We have developed a few demonstration applications, applets, and stylesheets (used as templates to map document type conversions). SpectroML will evolve as the ASTM task group refines and expands it to work across the breadth of molecular spectrometry and perhaps even beyond to atomic spectrometry and chromatography.

Purpose: To develop, document, and provide demonstration applications of a “web-aware” mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange.



The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. Over the past 15 years, several different interchange formats have been developed that allow data generated by different vendor systems of a given technique to be exchanged. For example, using JCAMP-DX, users of almost every commercial FT-infrared system can interchange their data. However, in the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange beyond the capabilities of these protocols. Today's analytical chemists need not only to interchange data generated within a specific technique, they need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. Users are now no longer content with just having “pictures” of their data (i.e., data plots). They need real

access to their data from within a report itself—they want to be able to expand plots, re-integrate peaks, overlay spectra, etc. without having to revert to the instrument or computer system that generated the original data. And, they don't want to have to purchase, learn, or even load proprietary programs to do this. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel that generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility.

Major Accomplishments: Over the past year, we have developed a “web-aware” mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectrometry data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectrometry and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers among ourselves, our colleagues at other national metrology institutes, and our NIST-Traceable Reference Material (NTRM) vendors. Limiting our scope solely to UV/visible data, a very limited subset of molecular spectrometry data, has allowed us to keep our initial project manageable.

The vocabulary of SpectroML was gleaned from the terminology, data dictionaries, and concepts embodied in existing standards, instrument software, and data interchange formats. Our objective was to take advantage of the large body of work that has been done in the field of spectrometric data interchange rather than re-inventing it. With this concept of reuse firmly in hand, we studied terminology definitions in normative standards, spectrometer operation and software manuals, and existing native and interchange formats, extracted the most useful parts of each, and organized it into five categories: file (header information), instrument (information about the instruments used), sample (information about the samples processed), measurement (information about the measurement process), and data (the result data values and information about their structure). The information in each category consists of two main parts: the “data,” which are the result data values from the experiment, and the “metadata,” which are descriptive data about the “data.” It is essential to keep both components together, because separately they both become useless.

We created SpectroML in the Extensible Markup Language (XML) and defined and documented it with both a DTD (Document Type Definition) and a schema. We have developed a few demonstration applications, applets, and stylesheets (used as templates to map document type conversions) and have used these demos to convince both instrument vendors and the ASTM E13 Molecular Spectrometry Committee that an XML-based approach is the way to go. At the 2001 Eastern Analytical Symposium, the ASTM E13 committee established a task group to support a markup language for molecular spectrometry based on SpectroML. Representatives from several major instrument companies have already signed on. SpectroML will evolve as the ASTM task group refines and expands it to work across the breadth of molecular spectrometry and perhaps even beyond to atomic spectrometry and chromatography.

Impact: The NIST-developed SpectroML, a “web-aware” mechanism, enables instrument-to-instrument, instrument-to-application, and application-to-application data interchange needed by today's analytical chemists. Representatives from several major instrument companies and the

ASTM E13 Molecular Spectrometry Committee support a markup language for molecular spectrometry based on SpectroML. NIST has developed demonstration applications but the impact of SpectroML will expand and evolve as an ASTM E13 task group and others refine and implement its use to applications across the breadth of molecular spectrometry and perhaps to other techniques.

Future Plans: At NIST, we are now developing the application programs to make SpectroML work with our and our NTRM vendors' instruments, so that we can begin to utilize it for UV/visible data interchange across the Internet.

UV-VIS-NIR Wavelength Standards for Transmission Measurements

CSTL Program: Chemical Characterization of Materials

Authors: *S.Choquette, D.L. Duewer, and D. Blackburn; L.M. Hanssen, and T. Early (844)*

Abstract: NIST continues to provide SRMs intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in the transmission mode. SRM 2065 was developed to extend the operational range of existing NIST NIR wavelength SRMs, complement the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provide a single glass optical standard useable from 334 nm to 1950 nm. A new SRM similar to SRM 2065 is currently being certified for wavelength verification and calibration of spectrometers operating in reflectance or quasi-reflectance (transflectance) modes.

Purpose: To provide SRMs intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in the transmission mode.

Major Accomplishments: Standard Reference Material[®] 2065 (SRM[®]) is a certified transfer standard intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in transmission mode. SRM 2065 is certified for the location of the center-of-gravity (COG) of twenty absorbance/transmittance bands, spanning the spectral region from 334 nm to 1946 nm. In addition, it is certified for the location of the COG of seven absorbance bands in the spectral region from 10,300 cm⁻¹ to 5130 cm⁻¹ at 4 cm⁻¹ resolution.

The NIR spectra of organic analytes are composed of overtone and combination bands from the primary vibrational transitions found in the mid-infrared. The extinction coefficients of these bands are 10 to 10000 times lower than those of the mid-infrared bands. This allows NIR spectroscopy to be used directly with native matrix samples, mostly without sample preparation and typically in real-time. A growing number of chemical industries especially agriculture, food, and pharmaceutical manufacturing are looking to the NIR for methods to replace traditional spectroscopic and wet chemical techniques. Because NIR bands are broad and overlap, full-spectrum, “chemometric” models are used to correlate the chemical composition of the desired analyte with its NIR spectrum. A critical requirement for NIR determinations is the calibration of the wavelength axis. NIST currently offers three NIR wavelength Standard Reference Materials, SRM 1920, SRM 2517, and SRM 2035. Only SRM 2035 is suitable for the calibration of NIR spectrometers operating in transmission throughout the entire NIR spectral range. SRM 2065 extends the operational range of SRM 2035 to include 13 additional bands between 334 nm and 805 nm. Accordingly, this SRM complements the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provides a single glass optical standard useable from 334 nm to 1950 nm [Figure 1].

The active element of SRM 2065 is a glass consisting of holmium, samarium ytterbium and neodymium oxides in a base glass containing oxides of lanthanum boron, silicon, and zirconium. The optical filter is 25 mm in diameter and 1.5 mm thick. This combination of the rare earth

oxide concentrations and filter thickness yields absorption bands between 0.1 and 0.6 absorbance units (AU) in the NIR and 0.2 to 1.3 AU (5% to 60% transmittance) in the UV-Visible. The standard was batch certified using a number of FT and dispersive spectrometers in both the analytical chemistry division and optical technology division. The 95% confidence interval for the NIR band locations is better than 0.3 cm^{-1} for the seven certified NIR bands and less than 0.3 nm for the 13 certified UV-Visible peaks.

Impact: A growing number of chemical industries especially agriculture, food, and pharmaceutical manufacturing are looking to the NIR for methods to replace traditional spectroscopic and wet chemical techniques. Because NIR bands are broad and overlap, full-spectrum, “chemometric” models are used to correlate the chemical composition of the desired analyte with its NIR spectrum. A critical requirement for NIR determinations is the calibration of the wavelength axis. SRM 2065 extends the operational range of existing NIST NIR wavelength SRMs, complements the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provides a single glass optical standard useable from 334 nm to 1950 nm.

Future Plans: We are currently certifying a new SRM similar to SRM 2065 for wavelength verification and calibration of spectrometers operating in reflectance or quasi-reflectance (transflectance) modes. SRM 2036 incorporates a glass very similar to that used in SRM 2065 into a sample holder containing a diffusely reflecting substrate (sintered polytetrafluoroethylene). This configuration will be useful for calibrating field spectrometers equipped with either integrating sphere or fiber probe sampling accessories

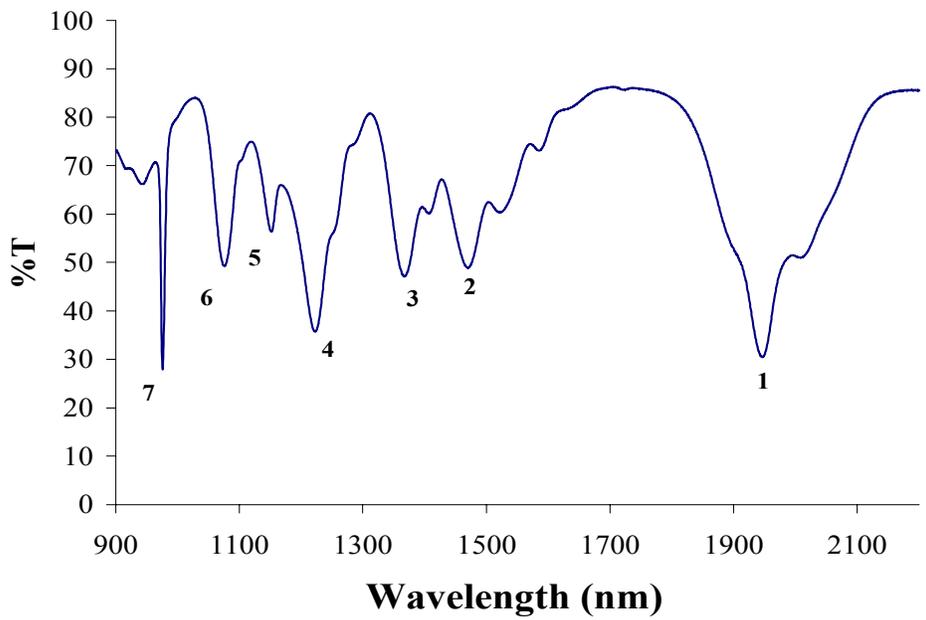
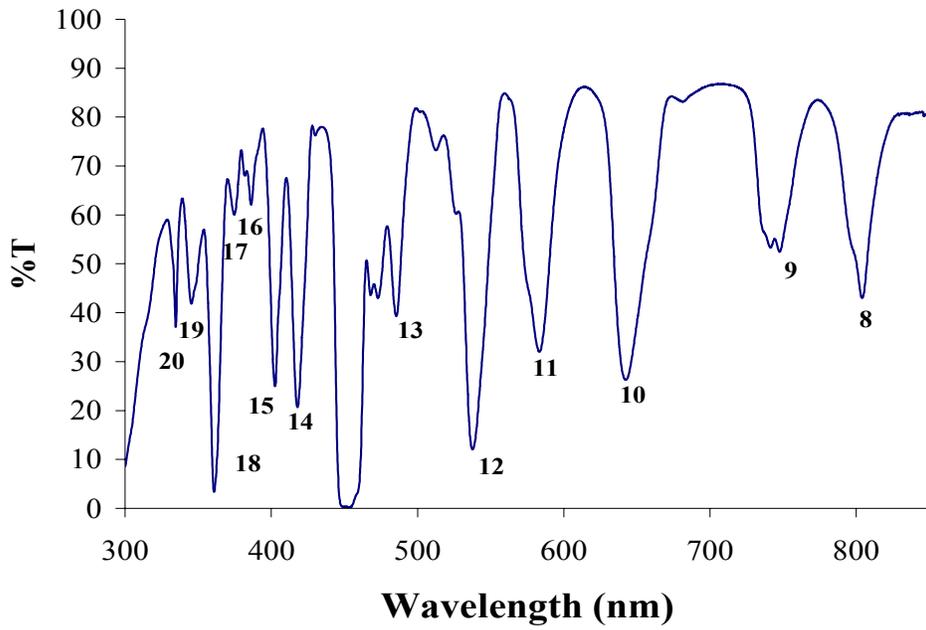


Figure 1. UV-Visible-NIR Spectra of SRM 2065

SRM 2241—A Relative Intensity Standard for Raman Spectroscopy using 785 nm Excitation

CSTL Program: Chemical Characterization of Materials

Authors: *S.J. Choquette, and D.H. Blackburn; E.S. Etz (837); and W.S. Hurst (836)*

Abstract: NIST SRMs for the calibration of the Raman intensity axis are needed to provide the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies. The first of a series of SRMs for the correction of the Raman spectral intensity is being issued this year. SRM 2241 is a certified transfer standard intended for the calibration of the spectral response function of a Raman spectrometer using 785 nm laser excitation. The active element of SRM 2241 is a fluorescent chromium-doped silicate glass that emits a broad, featureless fluorescence spectrum when excited with 785 nm laser radiation. It has been developed specifically to meet the needs of the analytical Raman community and is certified as an intensity standard for all common 785 nm excitation Raman spectroscopy systems employing macro- and micro-sampling configurations, as well as fiber-probe optical sampling. Because different materials will be needed for other laser excitation wavelengths, NIST is developing a series of Raman intensity correction SRMs for the popular excitation wavelengths. E.g., a uranium glass material is being qualified for Ar ion and doubled YAG laser excitations at 488nm/514nm and 532nm respectively (SRM 2242), a europium glass is being investigated for use with YAG lasers at 1064 nm (SRM 2243), and we are searching for a glass to use with HeNe at 633 nm (SRM 2244).

Purpose: NIST SRMs for the calibration of the Raman intensity axis are needed to provide the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies.

The first of a series of Standard Reference Materials[®] (SRM[®]s) for the correction of the Raman spectral intensity is being issued this year [Figure 1]. SRM 2241 is a certified transfer standard intended for the calibration of the spectral response function of a Raman spectrometer using 785 nm laser excitation. Using this standard allows the relative intensities of the bands observed in a Raman spectrum excited at 785 nm to be normalized to the intensity of a standard tungsten light source (white light). This, in turn, allows the direct comparison of spectra obtained on different instruments, the development of standard spectral libraries, and the verification of instrument performance. The active element of SRM 2241 is a fluorescent chromium-doped silicate glass that emits a broad, featureless fluorescence spectrum when excited with 785 nm laser radiation. It has been developed specifically to meet the needs of the analytical Raman community and is certified as an intensity standard for all common 785 nm excitation Raman spectroscopy systems employing macro- and micro-sampling configurations, as well as fiber-probe optical sampling. The fluorescence intensity of the SRM is calibrated using a certified NIST radiometric lamp standard.

Major Accomplishments: Recent advances in analytical Raman instrumentation, lasers, and detectors have revolutionized Raman spectroscopy. A variety of Raman spectroscopic techniques, employing both macro- and micro-sampling methods, are now well established in industry for process measurements and quality control. However, a general lack of accepted practices, standards, and spectral libraries hinders the acceptance of Raman spectroscopy, especially in regulated settings. While the calibration of the Raman frequency (or Raman shift, cm^{-1}) axis using pure materials and an existing ASTM standard is well accepted, techniques for calibration of the Raman intensity axis are not. Intensity calibrations of Raman spectra can be accomplished with certified white light sources, but this procedure has many drawbacks and provides numerous pitfalls to the uninitiated. These practical limitations are avoided by our approach using certified fluorescent glasses. The development of SRM 2241 and the future SRMs to be issued for other Raman laser excitation wavelengths has been closely coordinated with the Raman community made up of users from major chemical industries, instrument manufacturers, and the regulatory agencies. We have collaborated closely with the ASTM E13.08 Subcommittee on Raman Spectroscopy. For example, last year in conjunction with E13.08, we conducted an interlaboratory evaluation of the candidate glass for SRM 2241 that was critical to the determining the surface-finish characteristics and design of the holder for the glass.

The spectral characteristics and function of SRM 2241 have been characterized on three different and independent Raman systems. The detailed measurements leading to the certification of this SRM have addressed a number of experimental variables that have direct bearing on the application of this standard with different instrument designs—among these are the laser irradiance conditions employed, optical geometry and other aspects of the optical excitation and collection systems, variations of the spectral response of the luminescent glass with changes in ambient temperature, and departures from the exact (785 nm) laser wavelength. Part of the certification of this SRM entailed fitting a fourth-order polynomial to model the luminescence spectrum of the glass. The SRM user uses this polynomial equation to correct the band intensities in an acquired Raman spectrum to Raman intensities relative to white light. The mathematical uncertainties associated with these computational procedures determine the accuracy of the intensity correction. For SRM 2241, the accuracy is well within 10 percent bounds across the full Raman spectral range.

Impact: Having NIST SRMs for the calibration of the Raman intensity axis provides the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies.

Future Plans: Unfortunately, one intensity standard cannot provide intensity axis corrections for all Raman spectrometers. Different materials will be needed for other laser excitation wavelengths. We are committed to developing a series of Raman intensity correction SRMs for the popular excitation wavelengths. Accordingly, we are now in the process of qualifying a uranium glass material for Ar ion and doubled YAG laser excitations at 488nm/514nm and 532nm respectively (SRM 2242), are investigating a europium glass for use with YAG lasers at

1064 nm (SRM 2243), and are searching for a glass to use with HeNe at 633 nm and (and perhaps also diode lasers at 635 nm) (SRM 2244).



Figure 1. SRM 2241–Relative Intensity Correction Standard for Raman Spectroscopy: 785 nm Excitation.

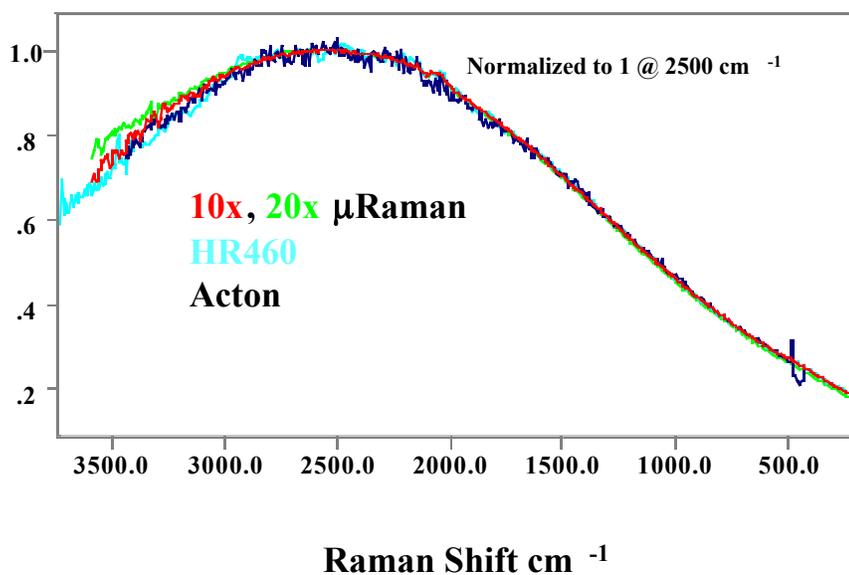


Figure 2. White light-corrected SRM 2241 on μRaman, HR460, and Acton Raman Systems

Measurement Tools and Standards for Forensic Chemistry

CSTL Program: Chemical Characterization of Materials

Authors: *Human Identity Testing Based on DNA: D.L. Duewer, and M.C. Kline*

Alcohol and Drugs of Abuse Testing: K.Phinney, M.J. Welch, L.T. Sniegowski, and S.S. Tai

Human Identification Based on Surface Components of Hair: B.A. Benner, and J.V. Goodpaster

Investigation of Handgun and Explosives Crime Scenes Based on Organic Residue Analysis: W.A. MacCrehan, and J.D. Secl

Chemical Weapons Treaty Verification: E. White, V.; W.G. Mallard (838); and E.S. Etz (837)

Abstract: Forensic chemical analyses have become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Currently in the forensic area, the Analytical Chemistry Division develops quantitative analytical techniques for the measurement of drugs in hair samples and for the determination of gunshot residue, examines DNA measurement technologies using statistical techniques to assure the quality of such results for identification and evidentiary purposes, develops and certifies SRMs for forensic analyses that are used to validate the accuracy of methods and provide traceability for measurements made in forensic laboratories around the world and includes activities in support of chemical weapons treaty verification activities.

Purpose: To develop, evaluate critically, and apply quantitative analytical chemistry techniques for forensic analyses and to develop analytical standards for the forensic community.

Chemical measurements often play a pivotal role in criminal investigations. Making the correct identification of a rapist, determining if suspicious white powder is truly methamphetamine, or evaluating whether a kidnapped child spent time in the trunk of a suspect's car are all examples of how reliable chemical measurements can be used to help fight crime. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Perhaps the greatest impact of our measurement technology, standards, and quality assurance efforts has been in gaining the widespread acceptance of human identification based on DNA "fingerprints". Three NIST Standard Reference Materials (SRMs) designed for validating different fingerprinting methods have been developed and are routinely used by the forensic

community. A recent NIST study reveals the importance of an accurate quantitation of DNA prior to PCR analysis.

CSTL has a long-term program to support accuracy in alcohol and drugs-of-abuse testing. Our activities include: development and maintenance of SRMs for alcohol use, urine drug testing and drug testing in hair. We have recently begun an effort to evaluate technologies used to screen substances for illicit drug content.

Research in our division has also pointed to a new idea in human identification based on surface components of the hair by evaluating the hydrocarbon profile of individual hairs. Fatty compounds such as fatty acid/ester/alcohols, and cholesterol enter the hair from the sweat glands, root blood supply, shampoos and environmental exposure. By extracting a single hair into the inlet of a gas chromatography/mass spectrometry system using coupled supercritical fluid extraction, it is possible to obtain a profile for an individual. Youngsters appear to have a set of age-dependant characteristics, providing a means of identifying hair from lost or missing children.

Another problem facing forensic chemists is in the investigation of handgun and pipe bomb crime scenes. Since the 80's, we have championed the idea of quantifying the gunpowder propellant and its stabilizer additives as a means of evaluating residues from the perpetrator or crime scene and associating them with ammunition/powder samples recovered from a suspect. To aid forensic laboratories in making accurate gunpowder additive determinations, we are currently preparing a NIST Reference Material for gunpowder composition.

In addition, CSTL makes a significant contribution to the fight against international terrorism by participating in the international effort in Chemical Weapons Treaty verification.

Major Accomplishments:

Human Identity Testing Based on DNA

Working together, the Biotechnology and Analytical Chemistry Divisions of CSTL help ensure that human identity testing based on DNA measurement technologies provide unequivocal information for forensic, human remains, and paternity investigations. As an active member of the Scientific Working Group for DNA Analysis Methods (SWGDM), we have contributed to the development of measurement technologies as well as the development of quality control and assurance tools. Starting in 1991, we have developed a series of Standard Reference Materials for evaluating and demonstrating the quality of various forms of DNA testing. We helped establish data validation criteria for the Federal Bureau of Investigation Laboratory's CODIS program. In collaboration with the Armed Forces Institute of Pathology's Department of Defense DNA Registry and others, we have evaluated the suitability of DNA archival media for long-term storage of blood stains at room temperature. Through a series of interlaboratory comparison studies now involving more than 80 DNA testing laboratories around the world, we are now evaluating the role that quantification of DNA has in the analysis of samples containing DNA from more than one source.

Current forensic human identity testing is largely based upon the identification of Short Tandem Repeat (STR) markers in DNA amplified via the Polymerase Chain Reaction (PCR). While PCR amplification has become quite routine, the amount of DNA used in the PCR reaction is widely recognized as a limiting factor. Commercially available STR typing systems are typically designed for amplification of about 1 ng of template DNA, with a stated tolerance of about a factor of two (from 0.5 to 2 ng). In a study of STR practice concluded in late 1999, we established that the expected among-laboratory standard deviation for estimating the quantity of DNA was itself about a factor of two [MSS1&MSS2]. Preliminary analysis of data from an interlaboratory study that will conclude in mid-October 2001 confirms this result [Figure 1] and suggests that it does indeed have consequences in the analysis of mixed-source DNA samples [Figure 2]. Too low an estimate of DNA quantity can lead to too much DNA being used in the amplification reaction (tending to increase the severity of amplification artifacts and thus complicates the analysis). Too high an estimate can lead to too little DNA in the reaction (tending to cause signals from minor source components to fall below established signal/noise thresholds).

DNA measurement technologies: <http://www.cstl.nist.gov/biotech/DNAtech/dnatech.html>

SWGDM: <http://www.for-swg.org/swgdamin.htm>

Standard Reference Materials

<http://srmcatalog.nist.gov/nist/products.nsf/all?SearchView&Query=dna&SearchOrder=4>

CODIS program: <http://www.fbi.gov/hq/lab/org/systems.htm>

Department of Defense DNA Registry: <http://www.afip.org/Departments/oafme/dna>

STRs: <http://www.cstl.nist.gov/biotech/strbase/>

MSS1&MSS2: Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. NIST Mixed Stain Studies #1 and #2: Interlaboratory Comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples. In press, J Forensic Sci. (web link available soon.)

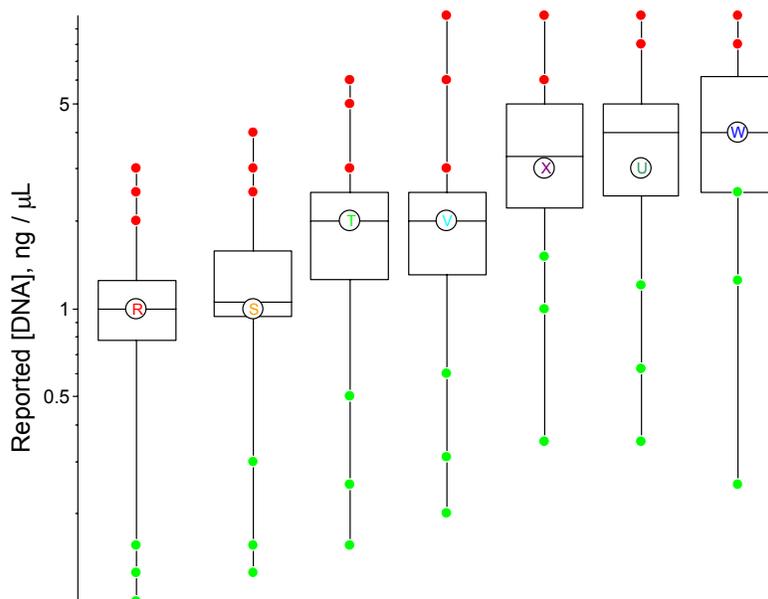
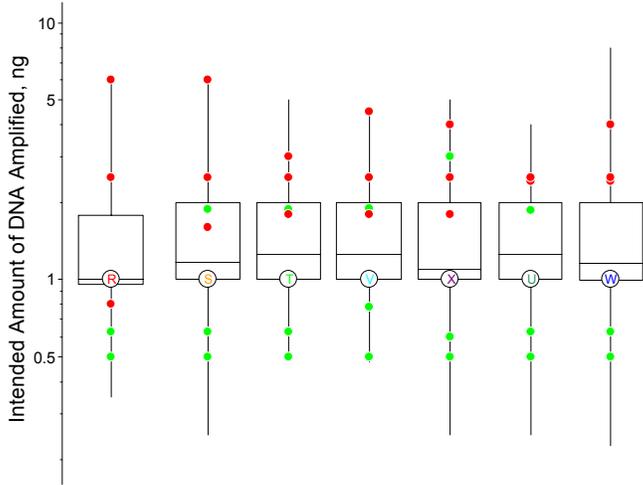


Figure 1. Figure DLD1
Distributions of Reported [DNA] Values

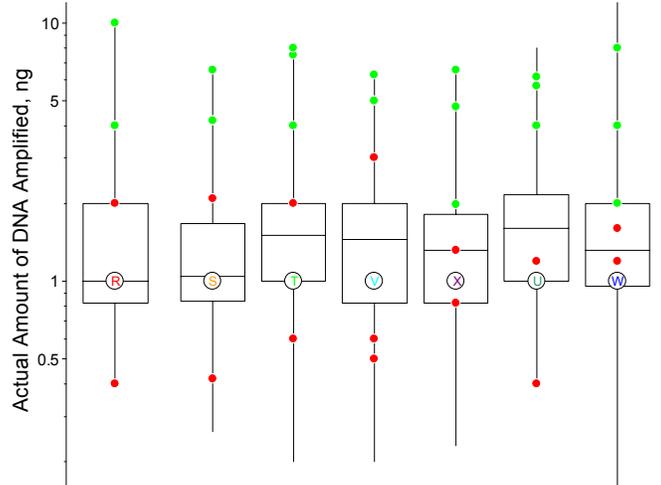
Box&whisker plot of reported [DNA] for seven DNA extracts distributed in the NIST-sponsored interlaboratory Mixed Stain Study #3. The “whiskers” span the lowest to the highest reported value for each extract. The “box” bounds the central 50% of the reported values; the horizontal line within the box denotes the interlaboratory median. The width of the box is proportional to the number of reported values. The open circles, labeled with the sample code, denote the designed [DNA] of each extract. The solid circles denote the same six participants, three each reporting extremely high (red) and low (green) [DNA].

For nearly all of the extracts, the interlaboratory median and the designed [DNA] are nearly identical. The cause(s) of the modest difference between the design and reported concentration for extract “U” is currently under investigation.

For all extracts, the central 50% of the participants report [DNA] that are within factors of 1.2 to 1.5 of the median. However, there are extreme values up to a factor of 8.0 from the median.



Intended Amount of DNA amplified
(Volume amplified)(Reported [DNA])



Actual Amount of DNA amplified
(Volume amplified)(Consensus [DNA])

Figure 2.

The left-hand Box&Whisker shows the distributions of the amounts of DNA that the participants *intended* to amplify, calculated as the product of their reported [DNA] and volume of extract added to the PCR reagents. Most participants intended to amplify one to two ng of DNA, although some (such as one of those reporting very low [DNA] results) chose to target somewhat more or somewhat less based upon their past experience with their analytical system.

The right-hand Box&Whisker shows the distributions of the amounts of DNA that the participants *actually* amplified, using the observed median to define the “true” [DNA] for each extract. The participants reporting extremely high [DNA] (red circles) tended to amplify much less than their target amount of DNA and thus tended not to “see” alleles from the minor components of the multiple-source extracts. The participants reporting extremely low [DNA] (green circles) tended to amplify much more than their target amount of DNA. This often excessive amount of template DNA tended to increase the number of “stutter” artifacts and thus complicated their analyses.

Alcohol and Drugs of Abuse Testing

Substance abuse continues to be a major problem in the U.S. The National Institutes of Health (NIH), estimated that the economic cost of alcohol and drug abuse was \$246 billion in 1992 (<http://www.nih.gov/news/pr/may98/nida-13.htm>), with alcohol abuse and alcoholism generated about 60 percent of the estimated costs (\$148 billion), while drug abuse and dependence accounted for the remaining 40 percent (\$98 billion). In Texas alone, economic costs associated with substance abuse were estimated to be \$19.3B in 1997 (<http://www.tcada.state.tx.us/research/economics/1997/>), with productivity losses and premature death the primary contributors to the cost.

To combat the deleterious effects of substance abuse and to discourage its spread, various governmental bodies at the local, state, and federal level as well as private industry employ various drug tests. Testing may involve analysis of blood or breath for alcohol consumption, analysis of body fluids and tissues for drugs of abuse or their metabolites, or analysis of substances suspected of being illicit materials. Subjects tested may include persons suspected of driving under the influence of drugs or alcohol, suspects in other crimes, people on probation, applicants for jobs, persons holding jobs, particularly in cases where public safety is involved, and even parents in custody disputes.

There are many potential consequences of a positive drug test for an individual, including imprisonment, loss of employment, etc, therefore it is critical that tests be accurate, with very low instances of false positives. NIST has a long-term program to support accuracy in substance abuse testing. The activities include development and maintenance of SRMs for alcohol use testing; development and maintenance of Standard Reference Materials for instrument calibration and/or method validation associated with urine and hair testing as well as evaluation of products used to screen substances for illicit drug content.

SRM 1828a consists of a series of aqueous solutions with known concentrations of ethanol. For more information see (<http://srmcatalog.nist.gov/>). This SRM is used to calibrate breathalyzer devices used to test for the presence of alcohol in the breath of drivers suspected of being intoxicated. Levels deemed to be above the legal limit vary between states and over time. Recently NIST verified the stability of the ethanol in this SRM and plans are underway to add new levels to better address the lower levels that many states are implementing.

Over the last decade, NIST developed a series of human urine based SRMs for drugs of abuse, particularly those drugs identified as substances to be tested for in the Federal workplace (http://workplace.samhsa.gov/frames/frame_fed.htm). These SRMs include:

SRM 1507b	Marijuana metabolite in freeze-dried urine
SRM 1508a	Benzoyllecgonine (cocaine metabolite) in freeze-dried urine
SRM 1511	Multidrug of abuse in freeze-dried urine
SRM 2381	Morphine and codeine in urine
SRM 2382	Morphine glucuronide in urine

More information can be found at (<http://srmcatalog.nist.gov/>). NIST regularly monitors the stability of these SRMs.

In recent years, considerable attention has focused on hair testing for drugs of abuse. Hair provides a long window of detection for drug use up to several months, as opposed to urine or blood testing which only show evidence for drug use over the previous few days. There are a number of controversial issues related to using hair analysis for drug testing, but a number of commercial laboratories are now regularly performing these analyses. Applicants for jobs at many private and public organizations now must submit a hair sample for drug testing as part of the screening process and many parolees are now also being tested in this manner.

As with urine drug testing, accuracy is critical for hair testing, considering the consequences of a positive test. NIST is developing two new drugs of abuse in human hair SRMs to provide laboratories with tools for evaluating the accuracy of their methods. A total of ten analytes were soaked into the two human hair materials. The concentrations of these analytes will be certified in the two materials, using NIST measurements involving GC/MS and LC/MS. These materials will be available in FY 02.

As part of a research effort in the separation and measurement of chiral compounds, NIST is investigating the measurement of chiral drugs in hair. For example, methamphetamine, a chiral compound, is an illicit substance produced in clandestine laboratories. The illicit material is the "d" enantiomer, while the "l" enantiomer is found in over-the-counter health products. A chiral separation is necessary to separate the illicit form from the legal form, which otherwise have identical chemical and physical properties. The goal of this research is to develop methods and standards to support critical chiral separations.

One new area for NIST involves the evaluation of spray reagents used to detect drugs of abuse. In routine police stops, substances are sometimes found that resemble drugs of abuse. The police need a quick means to determine if the substances may be drugs of abuse. Certain products are being marketed to police and perhaps, even to schools, for conducting such tests, with the warning that positive tests should be subjected to confirmatory testing. Because of concerns at the National Institute of Justice about the sensitivity and specificity of these products, NIST was asked to evaluate some of them. NIST is testing products for detecting cannabis, cocaine, and opiates for sensitivity and for reactions to non-drug materials used by people. This independent review will provide law enforcement personnel with valuable information as to the performance of such products.

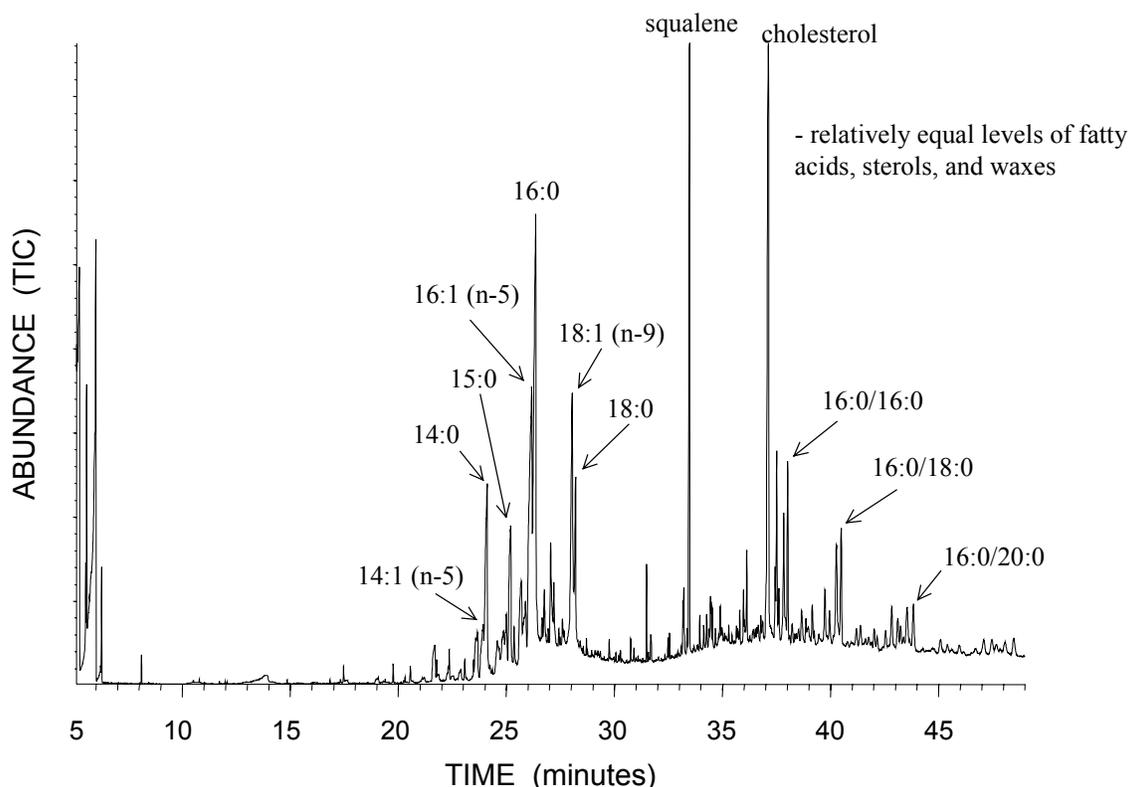
Human Identification Based on Surface Components of Hair

An ongoing study in our laboratory is the characterization of surface components of small samples of hair as a trace evidence analytical technique. Results from a preliminary study of hair samples from 20 volunteers using an on-line supercritical fluid extraction-gas chromatography/mass spectrometry (SFE-GC/MS) technique suggested that the chemical profile of surface components from an individual's hair may be used to distinguish him/her from another person. This analysis might provide complementary information to other microscopic and genetic techniques currently used in forensic laboratories.

Recently, macroscopic amounts (0.2 - 1.3 g) of a composite hair sample from a single subject (collected from age 10 - 18 y) were extracted using four different extraction techniques and four

different solvents. Of the techniques studied, pressurized solvent extraction was the most convenient and was amenable to sample sizes as small as 50 mg. Subsequent GC/MS analysis of the hair extracts has shown them to contain a complex mixture of lipids (see Figure below). The major components observed in the hair extracts were largely uniform across all solvents used for extraction, consisting of saturated and unsaturated free fatty acids from C₁₂ to C₁₈, cholesterol, in addition to squalene and larger waxes such as fatty acid esters. Consistent with the biological origin of the material, even carbon number fatty acids and esters predominated. One interesting result was that consistently higher levels of free fatty acids and cholesterol were observed in hair from pre-pubescent versus post-pubescent individuals.

Future work will compare the chemical profiles generated from extraction and analysis of small hair samples by SFE-GC/MS and pressurized solvent extraction with GC/MS analysis. Once the most effective analytical scheme is determined, hair samples from approximately 100 individuals will be characterized and the data examined for correlations based on age, gender, and ethnicity.



Pressurized solvent extraction (dichloromethane), 0.26 g of hair, asian male, 10-18 years (composite).

Investigation of Handgun and Explosives Crime Scenes Based on Organic Residue Analysis

For over ten years, CSTL has been developing new measurement technology and investigating standards issues for the detection of handgun and explosives crime. A comprehensive listing of our publications follows this text. Much of our recent research has been sponsored by the NIST Office of Law Enforcement Standards in collaboration with the National Institute of Justice. Our work is based on determining the organic additives in smokeless gunpowder residues. When gunpowder burns, it leaves minute traces as residues that can be collected as forensic evidence. For handgun crime, unburned particles of organic gunshot residues (OGSR) may be collected from the suspect's hands, hair or clothing. At explosive's crime scenes, forensic investigators look for residue particles amongst the debris from the explosion.

In our CSTL research on handgun residues, we have investigated the measurement of compounds added to smokeless powder as markers for residue detection. By measuring additives such as propellant nitroglycerin (NG) and stabilizers including diphenylamine (DPA), DPA's primary initial nitration product N-nitrosodiphenylamine (NnDPA), and ethyl centralite (EC – N, N'-diethyl-diphenylurea) the presence of gunpowder can be confirmed. A particular emphasis of the CSTL studies has been on quantitative determination of the additive content of gunpowder and its residues. In a model study, firing known gunpowder from handguns, we have investigated the composition of powder before and after firing. Overall, we have found that the residues are basically unburned gunpowder, reflecting well the composition before firing. The amount of NnDPA is often increased relative to DPA and the variability of the composition of the residues seems higher. For NG containing powders, we have found it useful to determine the ratio (P/S ratio) of propellant NG to total stabilizer (DPA+NnDPA or EC). Good P/S value agreement was found for handgun test firings of some commercial ammunition (J Forensic Sci 2001). We are currently working on the idea of collecting gunshot residues by combing shooter's hair with a fine toothed comb.

The analysis of smokeless powder is also important to the investigation of "improvised explosives" aka pipe bomb crime. In examination of the debris from an explosion, relatively intact powder grains can frequently be collected. Investigators then can categorize the residues by making dimensional and shape observations and to determine which of the additives is present. Accurate categorization relies on correct additive identification.

Recently, CSTL/NIST OLES sponsored an interlaboratory study of smokeless powder compositional and dimensional measurements. Besides our CSTL laboratory, the nineteen participants included laboratories from state and local, national, and international forensic laboratories. We sent two smokeless handgun reloading powder samples that had been previously characterized by NIST. Overall, participants accurately identified the primary additives. Some disagreement was noted for the determination of trace additives. Four participants also submitted quantitative results.

We are currently working to develop a smokeless powder reference material. With this NIST-traceable material, forensic laboratories can validate their analytical methods. The reference material could also be used as a proficiency test sample, verify a laboratory's capability in

explosives analysis. One state crime lab system used their successful participation in our interlaboratory study as partial requirements for their ASCLD-LAB accreditation for explosives determination.

Publication List

Separation and Identification of Gunshot and Explosive Constituents by Micellar Electrokinetic Capillary Electrophoresis; Northrop, D.M., MacCrehan, W.A., and Martire, D.E.; *Anal. Chem.* 63, 1038-1042 (1991).

Sample Collection, Preparation and Quantitation in the Micellar Electrokinetic Capillary Electrophoresis of Gunshot Residues; Northrop, D. M. and MacCrehan, W. A.; *J. Liq. Chromatogr.* 15, 1041-1063 (1992).

Smokeless Powder Residue Analysis by Capillary Electrophoresis; Northrop, D.M. and MacCrehan, W.A.; *NIJ Report 600-91* (1997).

Detection of Smokeless Powder Residues on Pipe Bombs by Micellar Electrokinetic Capillary Electrophoresis; Smith, K.D, McCord, B.R., MacCrehan, W.A., Mount, K. and Rowe, W.R.; in *Proceedings of the 5th International Symposium on Analysis and Detection of Explosives*, C.R. Midkiff, Ed. (1997).

Sampling Protocols for the Detection of Smokeless Powder Residues Using Capillary Electrophoresis; MacCrehan, W.A., Smith, K.D. and Rowe, W.R.; *J. For. Sci.* 43 (1), 119-124 (1998).

Detecting Organic Gunpowder Residues from Handgun Use; W. A. MacCrehan, K. M. Ricketts, R. A. Baltzersen, and W. F. Rowe; *Proceedings of the SPIE Conference on Enabling Technologies for Law Enforcement and Security*, K. Higgins, A.T. DePersia, and E.M. Carapezza, eds., SPIE Press, Bellingham, WA, (1998). pp. 116-124.

Detection of Smokeless Powder Residue on Pipe Bombs by Micellar Electrokinetic Capillary Electrophoresis; Smith, K.D., McCord, B.R., MacCrehan, W.A., Mount, K. and Rowe, W.F.; *J. For. Sci.* 44 (4), 789-794 (1999).

Comparing the Additive Composition of Smokeless Gunpowder and Handgun-fired Residues; Reardon, M.R., MacCrehan, W.A., and Rowe, W.F.; *J. For. Sci.* 45, 1197-1202 (2000).

NIST Efforts to Quality-Assure Gunpowder Measurements; MacCrehan, W.A., Reardon, M.R.; *Proceedings of the JANNAF 29th Propellant Development and Characterization Meeting*, CPIA Publication #697 - May 2000.

Investigating the Effect of Changing Ammunition on the Composition of Organic Additives in Gunshot Residue (OGSR); MacCrehan, W.A., Patierno, E.R., Duewer, D.L., and Reardon, M.R.; *J. For. Sci.* 45, 54-59 (2001).

Developing a Quantitative Extraction Technique for Determining the Organic Additives in Smokeless Handgun Powder; Reardon, M.R. and MacCrehan, W.A.; J. For. Sci. 2001; 46, 802-807.

Associating Gunpowder and Residues from Commercial Ammunition Using Compositional Analysis with the Propellant to Stabilizer Ratio (P/S); Reardon, M.R., MacCrehan, W.A., and Duewer, D.L.; J. For. Sci. 2001, *in press*.

A Preliminary Interlaboratory Study of Forensic Smokeless Powder Measurements; MacCrehan, W.A. and Reardon, M.R.; J. For. Sci. 2001; *in press*.

Testing a Little Gunpowder: An Interlaboratory Study of Smokeless Gunpowder Measurements; MacCrehan, W.A., Reardon, M.R., and Duewer, D.L.; *submitted to Anal. Chim. Acta* (2001)

Hot links:

Model study

<http://www.astm.org/cgi-bin/SoftCart.exe/jforensicsci/PAGES/3382.htm?L+mystore+prne5938>

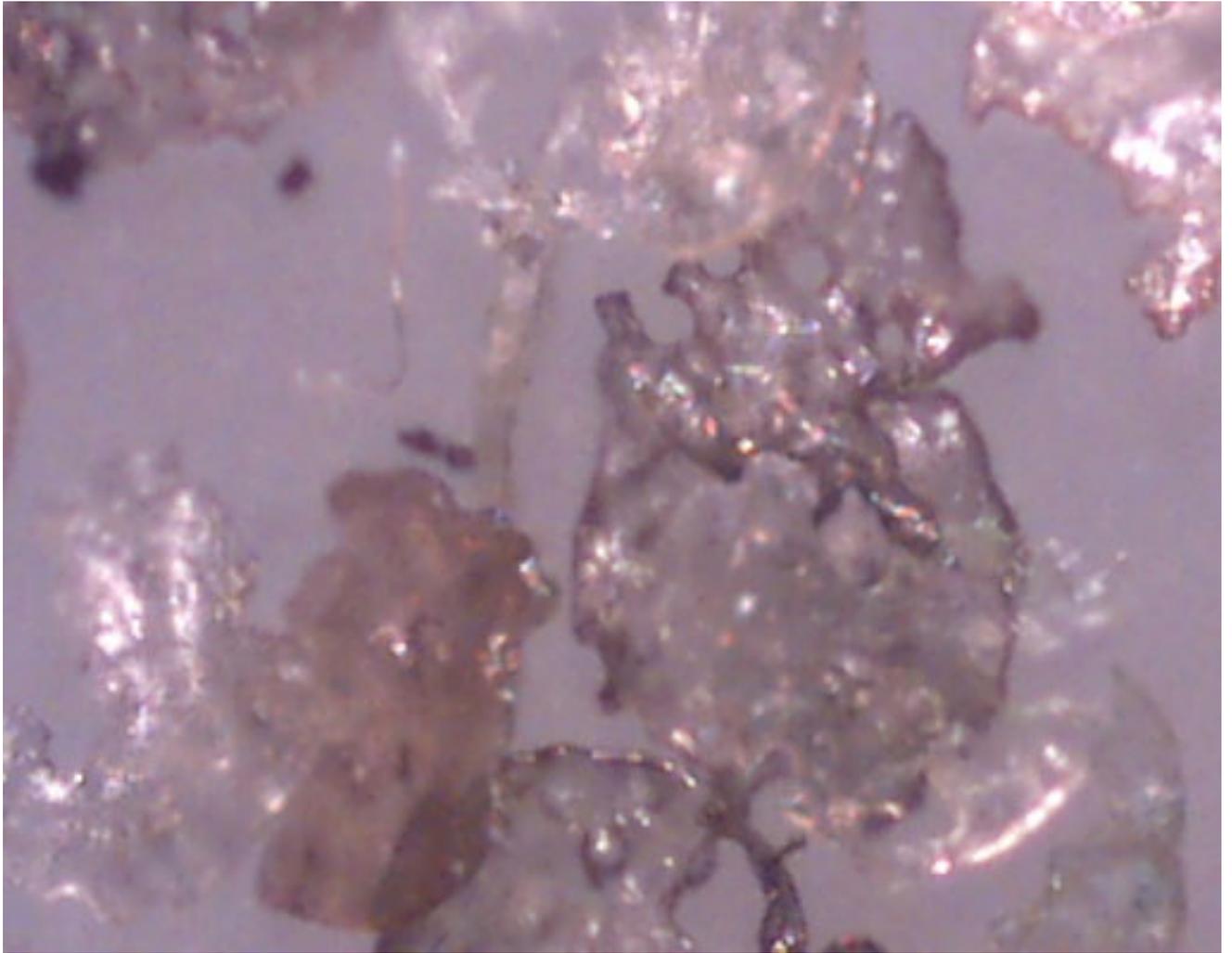
NIST Office of Law Enforcement Standards

<http://www.eeel.nist.gov/oles/>

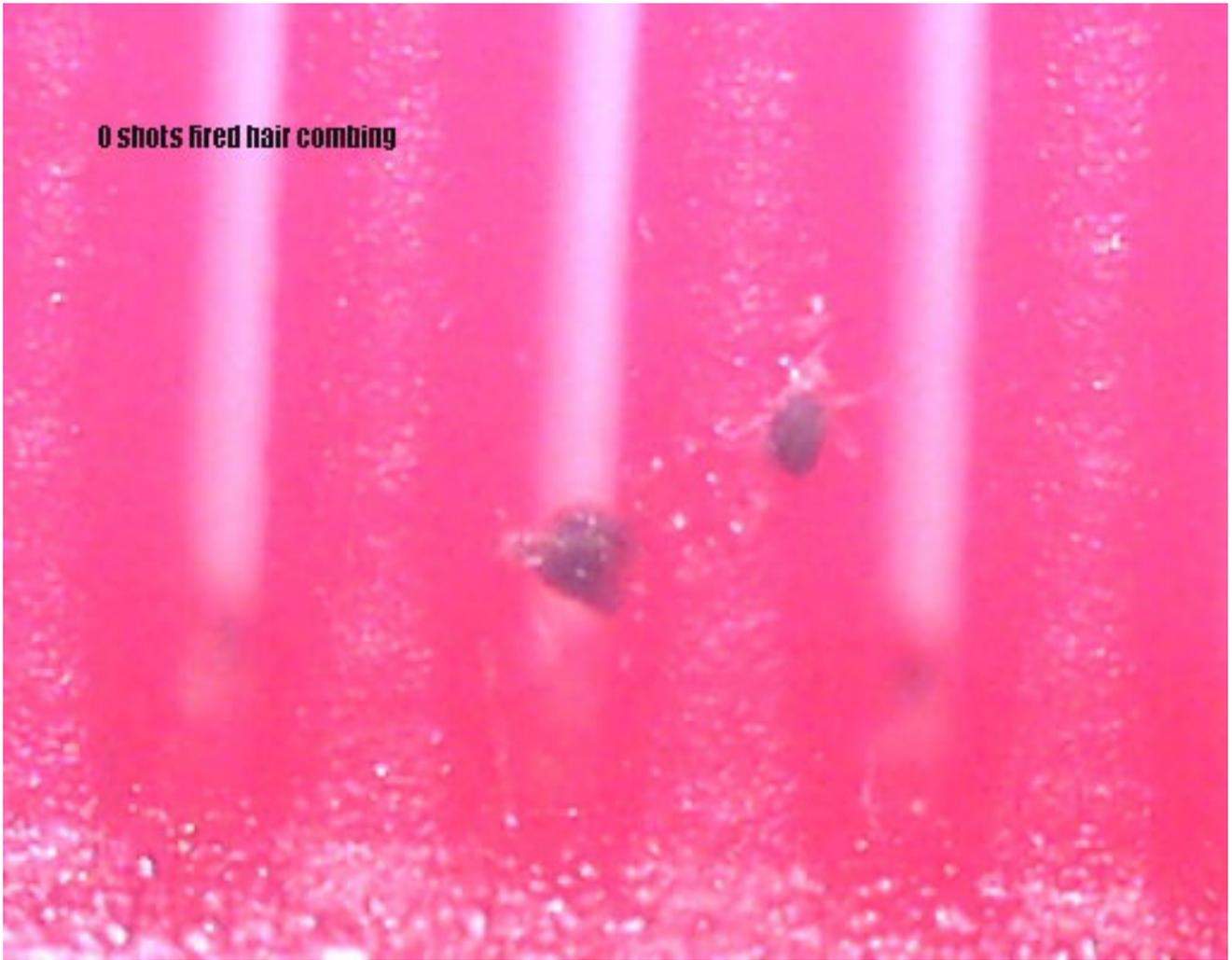
ASCLD-LAB

<http://www.ascl-d-lab.org/>

Organic gunshot residues



Combing shooter's hair



Chemical Weapons Treaty Verification



CSTL is taking a prominent role in the international effort to prevent the misuse of technology for the manufacture of chemical weapons. We are working with the Organization for the Prohibition of Chemical Weapons, whose mission is to administer the Chemical Weapons Convention (CWC). As members of the Validation Group for the Central Analytical Database, CSTL researchers from Divisions 837, 838, and 839 are responsible, along with representatives from other countries, for insuring the quality of the data added to the OPCW database. High quality data is essential to the correct identification of restricted or prohibited chemicals during inspection of chemical manufacturing facilities. There are several working groups within the Validation Group devoted to different chemical data types: infrared spectra, retention data for gas chromatography, and mass spectral data. CSTL experts support the United States Delegation to the OPCW with technical assistance on the drafting of documents relating to the taking and transfer of samples at inspection sites under the CWC. In addition, CSTL researchers provide direct onsite support for the consultations in The Hague.

The U.S. is one of the 143 signatory parties to the CWC, a global treaty bans chemical weapons. Issued in August 1994, the Convention reaffirmed principles and objectives of and obligations assumed under the Geneva Protocol of 1925, and the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction signed at London, Moscow and Washington in April 1972.

U.S. companies engaged in activities that involve certain chemicals may be required to submit reports to the Department of Commerce and may be subject to inspection by the OPCW.

Organization for the Prohibition of Chemical Weapons: <http://www.opcw.nl/>
Chemical Weapons Convention: <http://www.opcw.nl/guide.htm>

Impact: Chemical measurements often play a pivotal role in criminal investigations. Making the correct identification of a rapist, determining if suspicious white powder is truly methamphetamine, or evaluating whether a kidnapped child spent time in the trunk of a suspect's car are all examples of how reliable chemical measurements can be used to help fight crime. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Future Plans: The NIST Analytical Chemistry Division will continue its collaborations with the forensics community to assess needs and priorities for ACD development and critical evaluation of quantitative analytical chemistry techniques and to develop high priority analytical standards for the forensic community.